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**WO 01/34805 A2**

(54) Title: HUMAN VANILLOID RECEPTOR GENE

(57) Abstract: The present invention provides an isolated or purified polynucleotide that encodes human vanilloid receptor. Isoforms of human vanilloid receptor are also disclosed. The invention also provides methods of making recombinant human vanilloid receptor using the polynucleotides and host cells transformed with the polynucleotides.

## HUMAN VANILLOID RECEPTOR GENE

RELATED APPLICATIONS

This application claims priority to U.S. Application Serial Number 09/191,139 filed  
5 November 13, 1998.

TECHNICAL FIELD

The invention relates generally to polynucleotide sequences and polypeptide sequences  
10 encoded therefrom, more specifically, to vanilloid receptor genes and polypeptides encoded  
therefrom as well as methods which utilize these polypeptides for identifying compounds which  
modulate vanilloid receptors in human tissues.

BACKGROUND OF THE INVENTION

Vanilloid receptors are a class of ligand-gated ion channels defined by the natural  
15 ligands capsaicin, the active ingredient of hot peppers from plants of the genus *Capsium* and  
resiniferitoxin (RTX), an ultrapotent capsaicin analog found in the latex of *Euphorbia*  
*resinifera* (Holzer, Pharmacol. Rev. 43:143-201 [1991]). These receptors are involved in a  
variety of physiological processes including nociception, inflammation, regulation of body  
temperature, cardiovascular and bronchial systems, reflex bladder function and gastric  
20 mucosal defense mechanisms (Capsaicin in the study of pain, Wood ed., 1993, Academic  
Press).

The vanilloid receptor has been characterized as a cation permeable ion channel with  
the permeability of di- and mono-valent cations being  $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^{+} > \text{Na}^{+}$  (Bevan  
and Szolcsanyi, Trends Pharmacol. 11:330-333 [1990]). Activation of neuronal vanilloid  
25 receptors by capsaicin results in initial excitation resulting in pain perception while  
prolonged exposure results in analgesic effects most likely through a desensitization process  
(Szallasi, Gen. Pharmac. 25:223-243 [1994]). This biphasic response is characteristic for  
the other physiological responses to capsaicin, first described for thermoregulation in the  
hypothalamus (Jancsó-Gábor et al., J. Physiol. 208:449-459 [1970]). The rat vanilloid  
30 receptor VR1 has been cloned (Caterina et al., *Nature* **389**:816-824 [1997]).

Because of capsaicin's role in physiological processes, it would be useful to identify  
compounds, which modulate the activity of a human vanilloid receptor and/or its analogs.  
However, efforts to identify such compounds have been hampered by the lack of readily  
available human vanilloid receptors or cell lines expressing the human vanilloid receptor  
35 gene for use in screening assays. Although the rat vanilloid receptor is thought to be  
expressed exclusively in sensory neurons, human sensory neuron tissue is extremely  
difficult to obtain. Furthermore, no cell lines have been reported which endogenously  
express the human vanilloid receptor gene. Thus, there is a need for a simple, easy and cost  
effective means to obtain large quantities of human vanilloid receptors and/or a cell line  
40 which expresses such receptors.

The present invention solves this problem by providing reagents, such as human vanilloid receptors, polynucleotides (and polymorphic variants thereof) which encode for human vanilloid receptors and recombinant expression systems for large-scale production of said receptors. The invention also provides methodologies, such as assays, for identifying compounds which modulate the activity of human vanilloid receptors. Thus, the invention provides high throughput screening assays to identify new vanilloid receptor ligands for the treatment of various disease states including neuropathic pain, inflammation, arthritis, rhinitis, pruritus, bladder dysfunction, cluster headache, wound healing and psoriasis.

### SUMMARY OF THE INVENTION

The present invention provides an isolated or purified polynucleotide comprising a nucleotide sequence which encodes a human vanilloid receptor and fragments or complements thereof. Preferably, the nucleotide sequence is SEQ ID NO:1 or fragments thereof. More preferably, the nucleotide sequence is SEQ ID NO:1 from about nucleotide position 435 to about nucleotide position 3050. The invention further provides a polynucleotide comprising a nucleotide sequence which encodes a human vanilloid receptor having the sequence of SEQ ID NO:3.

In another aspect, the polynucleotide can be produced by recombinant techniques. A recombinant molecule comprises a nucleotide sequence that encodes a human vanilloid receptor and is contained within an expression vector. The expression vector may be either a prokaryotic or a eukaryotic vector. Preferred expression vectors are pCIneo and pACSG2. In a more preferred embodiment, the nucleotide sequence which encodes a human vanilloid receptor has the sequence SEQ ID NO:1 from about nucleotide position 435 to about nucleotide position 3050.

The present invention further provides a host cell transformed with said vector. The host cell is either a prokaryotic or eukaryotic cell.

The present invention also provides a polypeptide of a human vanilloid receptor or fragments thereof. In a preferred embodiment, the polypeptide has the amino acid sequence SEQ ID NO:3. The polypeptide can be produced by recombinant technology and provided in purified form.

In another aspect, the invention provides a method for producing a polypeptide which contains at least one human vanilloid receptor epitope, wherein the method comprises incubating host cells transformed with an expression vector comprising a nucleotide sequence which encodes a human vanilloid receptor. Preferably, the expression vector comprises a nucleotide sequence having the sequence SEQ ID NO:1 and fragments and complements thereof. More preferably, the nucleotide sequence has the sequence SEQ ID NO:1 from about

nucleotide position 435 to about nucleotide position 3050. Even more preferably, the nucleotide sequence encodes a human vanilloid receptor having sequence SEQ ID NO:3.

In another aspect, the invention provides a method for identifying compounds that modulate vanilloid receptor activity, comprising the steps of: (a) providing a host cell that expresses the vanilloid receptor polypeptide; (b) mixing a test compound with the cell; and (c) measuring either (i) the effect of the test compound on the cell expressing the receptor, or (ii) the binding of the test compound to the cell or to the receptor. The host cell of the method is either a prokaryotic or eukaryotic cell. Preferably in the method, the measurement of step (c)(ii) is performed by measuring a signal generated by a signal-generating compound or by measuring a signal generated by a radiolabeled ion, a fluorescent probe or an electrical current.

In yet another aspect, the invention provides a method for identifying a cytoprotective compound, comprising the steps of: (a) providing a cell that expresses a vanilloid receptor polypeptide or fragment thereof; (b) combining a test compound with the cell; and (c) monitoring the cell or cellular function for an indication of cytotoxicity. The host cell of the method is either a prokaryotic or eukaryotic cell. Preferably, the method comprises providing a cell which has an expression vector comprising a polynucleotide having the nucleotide sequence SEQ ID NO:1 from about nucleotide position 435 to about nucleotide position 3050 operably linked to control sequences that direct the transcription of the polynucleotide whereby the polynucleotide is expressed in a host cell. More preferably, one of the control sequences comprises an inducible promotor. Even more preferably, the cell is maintained in the presence of a substance which minimizes or blocks a cytotoxic effect on the cell.

In yet another aspect, the invention provides a method of treating an individual having a condition associated with vanilloid receptor modulation, comprising administering to the individual an effective amount of a compound that controls the gene expression of vanilloid receptor, in a pharmaceutically acceptable excipient.

In yet another embodiment, the invention provides a monoclonal antibody or a polyclonal antibody which specifically binds to human vanilloid receptor having amino acid sequence SEQ ID NO:3 or fragments thereof.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the alignment (default parameters of the FRAMEALIGN Program, Wisconsin Sequence Analysis Package, Version 9, Genetics Computer Group, Madison, WI) between the consensus sequence (upper line) of the overlapping human Incyte ESTs 1427917 (nt 1-227) and 3460342 (nt 32-270) with the corresponding amino acid sequence of the rat vanilloid receptor (bottom line, Caterina et al., 1997, *supra*). In this Figure, the upper line

corresponds to nucleotides 1696-1966 of SEQ ID NO:7. The bottom line corresponds to amino acid residues 500-589 of SEQ ID NO:4.

FIG. 2 shows the alignment (default parameters of the GAP Program, Wisconsin Sequence Analysis Package, Version 9, Genetics Computer Group, Madison, WI) between cDNA sequences of the human vanilloid receptor (hVR1, top line, SEQ ID NO:7) and rat vanilloid receptor 1 (rVR1, bottom line, SEQ ID NO:2, Caterina et al., 1997, *supra*). Vertical lines between the two sequences indicate identical nucleotides at those positions. Methionine initiation codons (ATG) and stop codons (TGA in top strand and TAA in bottom strand) are boxed. The DNA sequence identity of the rat cDNA from nucleotides (nt) 44-2730 and the human cDNA from nt 163-2874 is 82%.

FIG. 3 shows the multiple alignment (default parameters of the Pileup and Pretty Programs, Wisconsin Sequence Analysis Package, Version 10, Genetics Computer Group, Madison, WI) of the amino acid sequences of the hVR1 (SEQ ID NO:8), rVH1 (SEQ ID NO:4, Caterina et al., 1997, *supra*), human vanilloid receptor-like protein (hVR2, Caterina et al., Nature 398:436-441 1999, SEQ ID NO:15) and human vanilloid receptor 3 (hVR3, SEQ ID NO:3). The consensus sequence identifies any identical amino acid position shared by these 4 proteins. Boxed regions indicate the ankaryn repeats (position 239-270, 294-317 and 370-403), potential transmembrane domains (positions 471-493, 519-540, 555-574, 579-597, 621-640 and 703-730) and the poor-loop region (position 671-691).

FIG. 4 shows the polymorphic regions of the human vanilloid receptor determined by direct sequencing of the PCR product of human small intestine RNA. The Sequencher™ chromatogram tracings (Sequencher™ Version 3.0, Gene Codes Corp., Ann Arbor, MI) are shown with arrows identifying the double peaks consistent with polymorphic positions. Nt position 1605 contains either a C or a T while nt position 1952 contains an A or a G.

FIG. 5 shows the GAP analysis of hVR1 (bottom sequence, positions 301-3410 of SEQ ID NO:7) and hVR3 (top sequence, positions 1-3055 of SEQ ID NO:1) DNA sequences.

FIG. 6 shows the GAP analysis of the derived amino acid sequences of hVR1 (bottom sequence) and hVR3 (top sequence), SEQ ID NOs:8 and 3 respectively.

FIG. 7 shows a graphical representation of expression of hVR1 and hVR3 by quantitative RT-PCR (ABI Prism 7700) of total RNA isolated from human adrenal gland (lane 1), brain (lane 2), cerebellum (lane 3), fetal brain (lane 4), fetal liver (lane 5), heart (lane 6), kidney (lane 7), liver (lane 8), lung (lane 9), mammary gland (lane 10), pancreas (lane 11), placenta (lane 12), prostate (lane 13), salivary gland (lane 14), skeletal muscle (lane 15), small intestine (lane 16), spleen (lane 17), stomach (lane 18), testes (lane 19), thymus (lane 20), trachea (lane 21), uterus (lane 22), DRG (lane 23), bladder (lane 24) and HEK293 cells (lane 25) using primers specific for hVR1 and hVR3. The hatched bars represent samples from an additional experiment.

FIG. 8 shows the nucleotide sequence (SEQ ID NO:1) of human vanilloid receptor 3 and deduced amino acid sequence (SEQ ID NO:3).

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### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides isolated and purified polynucleotides that encode a human vanilloid receptor, fragments thereof, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process for making a human vanilloid receptor using those polynucleotides and vectors, and isolated and purified recombinant human vanilloid receptor and polypeptide fragments thereof.

Portions of the nucleic acid sequences disclosed herein are useful as primers for the reverse transcription of RNA or for the amplification of cDNA; or as probes to determine the presence of certain cDNA sequences in test samples. Also disclosed are nucleic acid sequences which permit the production of encoded polypeptide sequences which are useful as standards or reagents in diagnostic immunoassays, targets for pharmaceutical screening assays and/or as components or target sites for various therapies. Isolation of sequences from other portions of the vanilloid receptor gene can be accomplished by utilizing probes or PCR primers derived from these nucleic acid sequences, thus allowing additional probes and polypeptides of the genome of interest to be established.

The present invention also provides methods for assaying a test sample for products of a human vanilloid receptor gene, which comprises making cDNA from mRNA in the test sample, and detecting the cDNA as an indication of the presence of a human vanilloid receptor gene. The method may include an amplification step, wherein portions of the cDNA corresponding to the gene or fragment thereof is amplified. Methods also are provided for assaying for the translation products of mRNAs. Test samples which may be assayed by the methods provided herein include tissues, cells, body fluids and secretions. The present invention also provides reagents such as oligonucleotide primers and polypeptides which are useful in performing these methods. For example, the invention provides monoclonal and polyclonal antibodies directed against at least one epitope contained within the polypeptide sequences of the invention which are useful for diagnostic tests and for screening for diseases or conditions associated with abnormal vanilloid receptor production.

Although the physiological manifestations of abnormal vanilloid receptor expression are as yet unknown in humans or other mammals, we postulate that the vanilloid receptor may play a pathological role resulting from its abnormal expression. For example, Caterina et al. have shown that HEK293 cells transfected with the vanilloid receptor are killed within several hours of continuous exposure to capsaicin. Therefore, it is reasonable to postulate that the presence of

vanilloid receptor in certain body fluids where it is not normally found may be indicative of a disease state, the further progression of which could be monitored by assaying for vanilloid receptor in such fluids. A similar role is seen for myelin basic protein (MBP) which in the normal physiological state is a membrane bound protein and therefore not found in body fluids, but in disease states such as multiple sclerosis, it is released into cerebral spinal fluid. Furthermore, the presence of a polynucleotide or fragment thereof which encodes vanilloid receptor in tissues or body fluids where it is unexpected, may also be indicative of a disease condition, in the case, for example, where the disease was manifest by cellular degeneration.

Thus, the reagents and methods described herein may enable the identification of certain markers as indicative of abnormal vanilloid receptor expression and the information obtained therefrom may aid in the diagnosis, staging, monitoring, prognosis and/or therapy of diseases or conditions which may be associated with such expression. Test methods include, for example, probe assays which utilize the sequence(s) provided herein and which also may utilize nucleic acid amplification methods such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR); and hybridization. In addition, the nucleotide sequences provided herein contain open reading frames from which an immunogenic epitope may be found. Preferably, such an epitope is unique to the disease state or condition associated with the vanilloid receptor gene. The uniqueness of the epitope may be determined by its immunological reactivity with the polypeptide product encoded by such gene, and lack of immunological reactivity with tissue(s) from non-diseased patients. Methods for determining immunological reactivity are well-known and include but are not limited to, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), hemagglutination (HA), fluorescence polarization immunoassay (FPIA); chemiluminescent immunoassay (CLIA), and others; several examples of suitable methods are described herein.

### Definitions

All patents, patent applications and publications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the content clearly dictates otherwise.

Unless otherwise stated, the following terms shall have the following meanings:

“Purified product” refers to a preparation of the product, which has been isolated from the cellular constituents with which the product is normally associated, and from other types of cells, which may be present in the sample of interest.

The term “isolated” means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same

polynucleotide or DNA or polypeptide, which is separated from some or all of the coexisting materials in the natural system, is isolated. Such a polynucleotide could be part of a vector and/or such a polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

5       The term "polynucleotide" as used herein means a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double- and single-stranded DNA, as well as, double- and single-stranded RNA. It also includes modifications, such as methylation or capping, and unmodified forms of the polynucleotide.

10       A "vanilloid receptor variant" refers to an isolated vanilloid receptor polynucleotide sequence having at least 83%, more preferably, at least 90% and even more preferably, at least 95% global sequence identity over a length of a vanilloid receptor polynucleotide, to vanilloid receptor polynucleotides disclosed herein. "Percent identity" is determined using the default parameters of the GAP program, Wisconsin Sequence Analysis Package, Version 9, Genetics  
15   Computer Group, Madison, WI).

      A "polynucleotide fragment derived from" a designated sequence refers to a polynucleotide sequence which is comprised of a sequence of approximately at least about 6 nucleotides, is preferably at least about 8 nucleotides, is more preferably at least about 10, is more preferably at least about 12 nucleotides, is more preferably at least about 15 and even  
20   more preferably is at least about 20 nucleotides corresponding, i.e., identical to or complementary to, a region of the designated nucleotide sequence. The sequence may be complementary to or identical to a sequence which is unique to a particular polynucleotide sequence as determined by techniques known in the art. Comparisons to sequences in databanks, for example, can be used as a method to surmise the uniqueness of a designated  
25   sequence. Regions from which sequences may be derived include but are not limited to regions encoding specific epitopes, as well as non-translated and/or non-transcribed regions.

      The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest under study, but may be generated in any manner, including but not limited to chemical synthesis, replication, reverse transcription or transcription, which is  
30   based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived; as such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use.

35       When referring to a nucleic acid fragment, such a fragment is considered to "selectively hybridize" or to "selectively bind" to a polynucleotide or variants thereof disclosed herein, if, within the linear range of detection, the hybridization results in a



stronger signal relative to the signal that results from hybridization of the fragment to an equal amount of a second polynucleotide. A signal which is "stronger" than another is one which is measurable over the other by the particular method of detection. Methods for hybridizing and detecting polynucleotides are well known to those of ordinary skill in the art.

Also, when referring to a nucleic acid fragment, such a fragment is considered to hybridize under selective hybridization conditions if it selectively hybridizes under (i) typical hybridization and wash conditions, such as those described, for example, in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, second edition, (1989), Cold Spring Harbor, N.Y. and Nucleic Acid Hybridization: A Practical Approach, editors B.D. Hames and S.J. Higgins, (1985) Oxford; Washington D.C.; IRL Press), where preferred hybridization conditions are those of lesser stringency and more preferred, higher stringency; or (ii) standard PCR conditions (Saiki, R.K. *et al.* (1988) Science. 239:487-491) or "touch-down" PCR conditions (Roux, K.H., (1994), Biotechniques, 16:812-814).

"A sequence corresponding to a cDNA" means that the sequence contains a polynucleotide sequence that is identical to or complementary to a sequence in the designated DNA. The degree (or "percent") of identity or complementarity to the cDNA will be approximately 50% or greater, will preferably be at least about 70% or greater, and more preferably will be at least about 90% or greater. The sequence that corresponds will be at least about 50 nucleotides in length, will preferably be about 60 nucleotides in length, and more preferably, will be at least about 70 nucleotides in length. The correspondence between the gene or gene fragment of interest and the cDNA can be determined by methods known in the art, and include, for example, a direct comparison of the sequenced material with the cDNAs described, or hybridization and digestion with single strand nucleases, followed by size determination of the digested fragments.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and more preferably, less than about 90% of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density. Thus, "purified polypeptide" means a polypeptide of interest or fragment thereof which is essentially free, that is, contains less than about 50%, preferably less than about 70%, and more preferably, less than about 90%

of cellular components with which the polypeptide of interest is naturally associated. Methods for purifying are known in the art.

The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, i.e., peptide nucleic acid analog (PNA) or morpholino analog (MA) which can be used to identify specific DNA or RNA present in samples bearing the complementary sequence.

The term "primer" denotes a specific oligonucleotide sequence complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence and serve as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase or reverse transcriptase.

"Polypeptide" as used herein indicates a molecular chain of amino acids and does not refer to a specific length of the product. Thus, peptides, oligopeptides and proteins are included within the definition of polypeptide. This term, however, is not intended to refer to post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like.

A "recombinant polypeptide" as used herein means at least a polypeptide which by virtue of its origin or manipulation is not associated with all or a portion of the polypeptide with which it is associated in nature and/or is linked to a polypeptide other than that to which it is linked in nature. A recombinant or derived polypeptide is not necessarily translated from a designated nucleic acid sequence. It also may be generated in any manner, including chemical synthesis or expression of a recombinant expression system.

The term "synthetic peptide" as used herein means a polymeric form of amino acids of any length, which may be chemically synthesized by methods well-known to those of ordinary skill in the art. These synthetic peptides are useful in various applications.

A vanilloid receptor polypeptide, as used herein, refers to polypeptide having at least 87%, more preferably at least 90%, and even more preferably at least 95% global sequence identity over a length of a vanilloid receptor polypeptide, to vanilloid receptor polypeptides disclosed herein. A most preferred vanilloid receptor polypeptide is SEQ ID NO:3. Two other preferred vanilloid receptor polypeptides have essentially identical sequences to SEQ ID NO:3 with the exception that in one preferred polypeptide, at residue 469, threonine is replaced by isoleucine and in the second preferred polypeptide, at residue 586, isoleucine is replaced with valine.

A "polypeptide or amino acid sequence derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence or a portion thereof wherein the portion consists of at least 3 to 5 amino acids, and more preferably at least 8 to 10 amino acids, and even more preferably 15 to 20 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence.

As used herein, the term "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. The term "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" can be determined between the compared polypeptide sequences. Further, the polypeptide or amino acid sequence may preferably have at least 90% similarity, more preferably about 95% similarity and most preferably about 98% similarity to a polypeptide or amino acid sequence of a human vanilloid receptor.

The percent identity of two sequences, whether nucleic acid or peptide sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Schwartz, R.M., and Dayhoff, M.O. Matrices for detecting distant relationships, (in) Atlas of Protein Sequence and Structure, 5 suppl.3:353-358, (Nat. Biomed. Res. Found., Washington D.C.), 1978. and normalized by Gribskov, Nucl. Acids Res. 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic acid and peptide sequences is provided by the Genetics Computer Group (Madison, WI) in their GAP utility application. The default parameters for this method are described in the Wisconsin Sequence analysis Package, Program Manual, Version 9 (available from Genetics Computer Group, Madison, WI). Other equally suitable programs for calculating the percent identity or similarity between sequences are generally known in the art.

Other techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded therein, and comparing this to a second amino acid sequence.

"Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transferred DNA, and include the original progeny of the original cell which has been transfected.

As used herein "replicon" means any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

A "vector" is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoter, ribosomal binding site and terminators; in eukaryotes, such control sequences generally include promoters, terminators and, in some instances, enhancers. The term "control sequence" thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

"Operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "open reading frame" or "ORF" refers to a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5' -terminus and a translation stop codon at the 3' -terminus. A coding sequence can include, but is not limited to, mRNA, cDNA, and recombinant polynucleotide sequences.

The term "sense strand" or "plus strand" (or "+") as used herein denotes a nucleic acid that contains the sequence that encodes the polypeptide. The term "antisense strand" or "minus strand" (or "-") denotes a nucleic acid that contains a sequence that is complementary to that of the "plus" strand.

An "Expressed Sequence Tag" or "EST" refers to the partial sequence of a cDNA insert which has been made by reverse transcription of mRNA extracted from a tissue, followed by insertion into a vector.

A "transcript image" refers to a table or list giving the quantitative distribution of ESTs in a library and represents the genes active in the tissue from which the library was made.

The term "immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptide(s) which also are present in and are unique to the designated polypeptide(s). Immunological identity may be determined by antibody binding and/or competition in binding. These techniques are known to those of ordinary skill in the art and also are described herein. The uniqueness of an epitope also can be surmised by computer searches of known data banks, such as GenBank, for the polynucleotide sequences which encode the epitope, and by amino acid sequence comparisons with other known proteins.

As used herein, "epitope" means an antigenic determinant of a polypeptide. Conceivably, an epitope can comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids, and more usually, it consists of at least eight to ten amino acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

A "conformational epitope" is an epitope that is comprised of specific juxtaposition of amino acids in an immunologically recognizable structure, such amino acids being present on the same polypeptide in a contiguous or non-contiguous order or present on different polypeptides.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The methods for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

As used herein, the term "immunogenic polypeptide containing an epitope of interest" means naturally occurring polypeptides of interest or fragments thereof, as well as polypeptides prepared by other means, for example, by chemical synthesis or the expression of the polypeptide in a recombinant organism.

The terms "transformation" refers to the insertion of an exogenous polynucleotide into a prokaryotic or yeast host cell, irrespective of the method used for the insertion. Generally, the term "transfection" is used with respect to insertion of an exogenous polynucleotide into a eukaryotic host cell. The processes for achieving transformation and/or transfection are well known to those of ordinary skill in the art and include such techniques as direct uptake, transduction, f-mating and electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

"Treatment" refers to prophylaxis and/or therapy.

The term "individual" as used herein refers to vertebrates, particularly members of the mammalian species and includes but is not limited to domestic animals, sports animals, primates and humans; more particularly the term refers to humans.

The term "test sample" refers to a component of an individual's body which is the source of the analyte (also referred to "target" or "marker"). These components include antibodies and antigens and are well known in the art. These test samples include biological samples which can be tested by the methods of the present invention described herein and

include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitor-urinary tracts, tears, saliva, milk, white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; fixed tissue specimens; and fixed cell specimens.

5 "PNA" denotes a "peptide nucleic acid analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. "MA" denotes a "morpholino analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841, which is incorporated herein by reference. PNAs are neutrally charged moieties which can be directed  
10 against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and resistance to enzymatic degradation which is present in methods utilizing DNA or RNA. These PNAs can be labeled with such signal  
15 generating compounds as fluorescein, radionucleotides, chemiluminescent compounds, and the like. PNAs or other nucleic acid analogs such as MAs thus can be used in assay methods in place of DNA or RNA. Although assays are described herein utilizing DNA probes, it is within the scope of the routine that PNAs or MAs can be substituted for RNA or DNA with appropriate changes if and as needed in assay reagents.

20 "Analyte," as used herein, is the substance to be detected which may be present in the test sample. The analyte can be any substance for which there exists a naturally occurring specific binding member (such as, an antibody), or for which a specific binding member can be prepared. Thus, an analyte is a substance that can bind to one or more specific binding  
25 members in an assay. "Analyte" also includes any antigenic substances, haptens, antibodies, and combinations thereof. As a member of a specific binding pair, the analyte can be detected by means of naturally occurring specific binding partners (pairs) such as the use of intrinsic factor protein as a member of a specific binding pair for the determination of Vitamin B12, the use of folate-binding protein to determine folic acid, or the use of a lectin as a member of a specific binding pair for the determination of a carbohydrate. The analyte can include a protein,  
30 a peptide, an amino acid, a nucleotide target, and the like.

The present invention provides assays which utilize specific binding members. A "specific binding member," as used herein, is a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. Therefore, in addition to antigen and antibody  
35 specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like.

Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments, both monoclonal and polyclonal, and complexes thereof, including those formed by recombinant DNA molecules.

The term "hapten," as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

A "capture reagent," as used herein, refers to an unlabeled specific binding member which is specific either for the analyte as in a sandwich assay, for the indicator reagent or analyte as in a competitive assay, or for an ancillary specific binding member, which itself is specific for the analyte, as in an indirect assay. The capture reagent can be directly or indirectly bound to a solid phase material before the performance of the assay or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample.

The "indicator reagent" comprises a "signal-generating compound" ("label") which is capable of generating and generates a measurable signal detectable by external means, conjugated ("attached") to a specific binding member. The indicator reagent can be a member of any specific binding pair including hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme, and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to polypeptide of interest as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay. When describing probes and probe assays, the term "reporter molecule" may be used. A reporter molecule comprises a signal generating compound as described hereinabove conjugated to a specific binding member of a specific binding pair, such as carbazol or adamantane.

The various "signal-generating compounds" (labels) contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums and luminol, radioactive elements, and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

"Solid phases" ("solid supports") are known to those in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells, and

Duracytes® (red blood cells "fixed" by pyruvic aldehyde and formaldehyde, available from Abbott Laboratories, Abbott Park, IL) and others. The "solid phase" is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and Duracytes® are all suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase", as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, Duracytes® and other configurations known to those of ordinary skill in the art.

It is contemplated and within the scope of the present invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structure generally are preferred, but materials with gel structure in the hydrated state may be used as well. Such useful solid supports include but are not limited to nitrocellulose and nylon. It is contemplated that such porous solid supports described herein preferably are in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1mm. The pore size may vary within wide limits, and preferably is from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surface of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Other suitable solid supports are known in the art.

### Reagents

The present invention provides reagents such as polynucleotide sequences derived from a human vanilloid receptor gene, polypeptides encoded therein, and antibodies produced from these polypeptides. The present invention also provides reagents such as oligonucleotide



fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides. For example, selected vanilloid receptor-derived polynucleotides can be used in the methods described herein for the detection of normal or altered gene expression.

5           The present invention also provides methods, in particular, recombinant methodologies using polynucleotide sequences disclosed herein, for making human vanilloid receptors in high yield as well as methods to identify compounds which modulate (i.e. activate or repress) the activity of such receptors. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably purified.

10           Furthermore, the polynucleotides disclosed herein, their complementary sequences or fragments of either can be used in assays to detect, amplify or quantify genes, cDNAs or mRNAs encoding human vanilloid receptor. They also can be used to identify an entire or partial coding region which encodes for a vanilloid receptor polypeptide. They further can be provided in individual containers in the form of a kit for assays, or provided as individual  
15           compositions. If provided in a kit for assays, other suitable reagents such as buffers, conjugates and the like may be included.

          The polynucleotide(s) may be in the form of mRNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded may be  
20           the coding (sense) strand or non-coding (antisense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA provided herein.

          This polynucleotide may include only the coding sequence for the polypeptide, or the  
25           coding sequence for the polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence, or the coding sequence for the polypeptide (and optionally additional coding sequence) and non-coding sequence, such as a non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide.

          In addition, the invention includes variant polynucleotides containing modifications  
30           such as polynucleotide deletions, substitutions or additions; and any polypeptide modification resulting from the variant polynucleotide sequence. A polynucleotide of the present invention also may have a coding sequence which is a naturally occurring allelic variant of the coding sequence provided herein.

          In addition, the coding sequence for the polypeptide may be fused in the same reading  
35           frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence

is a preprotein and may have the leader sequence cleaved by the host cell to form the form of the polypeptide. The polynucleotides may also encode for a proprotein which is the protein plus additional 5' amino acid residues. A protein having a prosequence is a proprotein and may in some cases be an inactive form of the protein. Once the prosequence is cleaved an active protein remains. Thus, the polynucleotide of the present invention may encode for a protein, or for a protein having a prosequence or for a protein having both a presequence (leader sequence) and a prosequence.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pProEx1 (Life Technologies, Gaithersburg, MD) vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein. See, for example, I. Wilson, *et al.*, Cell 37:767 (1984).

It is contemplated that polynucleotides which encode a human vanilloid receptor will be considered to hybridize to the sequences provided herein if there is at least 60%, more preferably at least 70% and even more preferably at least 80%, identity between the polynucleotide and the sequence.

The present invention further provides human vanilloid receptor polypeptides which have the deduced amino acid sequences as provided herein, as well as fragments, analogs and derivatives of such polypeptides. The polypeptides of the present invention may be recombinant polypeptides, natural purified polypeptides or synthetic polypeptides. The polypeptides, fragments, derivatives or analogs of the human vanilloid receptor may be those in which one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence.

Thus, a polypeptide of the present invention may have an amino acid sequence that is identical to that of the naturally occurring polypeptide or that is different by minor variations due to one or more amino acid substitutions. The variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine or

threonine with serine. In contrast, variations may include nonconservative changes, eg, replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without changing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR Inc., Madison, WI).

A human vanilloid polypeptide is believed to be substantially encoded by or within SEQ ID NO:1 or SEQ ID NO:7. The minimum polypeptide sequence necessary for ligand binding, however, can be determined by routine methods. The sequence, for example, may be truncated at either end by treating an appropriate expression vector with an exonuclease (after cleavage at the 5' or 3' end of the coding sequence) to remove any desired number of base pairs. The resulting coding polynucleotide is then expressed and the sequence determined. In this manner the binding activity may be correlated with the amino acid sequence: a limited series of such experiments (removing progressively greater numbers of base pairs) determines the minimum internal sequence necessary for ligand-binding activity.

The vanilloid receptor polypeptides may be naturally purified products expressed from a high expressing cell line, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture) as described above. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. The polypeptides of the invention may also include an initial methionine amino acid residue. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers or produced by cell-free translation systems using RNAs derived from the DNA constructs of the present invention.

The present invention also provides an antibody produced by using a purified vanilloid receptor gene polypeptide of which at least a portion of the polypeptide is encoded by a vanilloid receptor gene polynucleotide selected from the polynucleotides provided herein. These antibodies may be used in the methods provided herein for the detection of vanilloid receptor polypeptides in test samples. The antibody also may be used for therapeutic purposes, for example, in neutralizing the activity of a vanilloid receptor polypeptide in conditions associated with its altered or abnormal expression. The antibody may also be used to detect an accessory protein or proteins by immunoprecipitation of protein complexes.

#### Probe Assays

The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. For example, such probes can be used

in Fluorescent In Situ Hybridization (FISH) technology to perform chromosomal analysis, and used to identify vanilloid receptor structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR-generated and/or allele specific oligonucleotide probes, allele specific amplification or by direct  
5 sequencing. Probes also can be labeled with radioisotopes, directly- or indirectly- detectable haptens, or fluorescent molecules, and utilized for in situ hybridization studies to evaluate the mRNA expression of the gene comprising the polynucleotide in fixed tissue specimens or cells.

The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The  
10 design of such probes for optimization in assays is within the skill of the routineer. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multigene family or in related species like mouse and man.

15 The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers are employed in excess to hybridize at the outside ends of complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation  
20 from the original target strand. New primers then hybridize to the target sequences and are extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in U.S. patents 4,683,195 and 4,683,202.

The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and second) and two secondary  
25 (third and fourth) probes, all of which are employed in molar excess to a target. The first probe hybridizes to a first segment of the target strand and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3'hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary)  
30 probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary,  
35 secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more

completely in EP-A- 320 308 to K. Backman published June 16, 1989 and EP-A-439 182 to K. Backman *et al.*, published July 31, 1991, both of which are incorporated herein by reference.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770, or reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R. L. Marshall, *et al.*, *PCR Methods and Applications* 4: 80-84 (1994).

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described in *PNAS USA* 87:1874-1878 (1990) and also described in *Nature* 350 (No. 6313):91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G. T. Walker *et al.*, *Clin. Chem.* 42:9-13 (1996)) and European Patent Application No. 684315; and target mediated amplification, as described by PCT Publication WO 9322461.

In one embodiment, the present invention generally comprises the steps of contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method herein provided are labeled with capture and detection labels wherein probes are labeled with one type of label and primers are labeled with the other type of label. Additionally, the primers and probes are selected such that the probe sequence has a lower melt temperature than the primer sequences. The amplification reagents, detection reagents and test sample are placed under amplification conditions whereby, in the presence of target sequence, copies of the target sequence (an amplicon) are produced. In the usual case, the amplicon is double stranded because primers are provided to amplify a target sequence and its complementary strand. The double stranded amplicon then is thermally denatured to produce single stranded amplicon members. Upon formation of the single stranded amplicon members, the mixture is cooled to allow the formation of complexes between the probes and single stranded amplicon members.

As the single stranded amplicon sequences and probe sequences are cooled, the probe sequences preferentially bind the single stranded amplicon members. This finding is counterintuitive given that the probe sequences are generally selected to be shorter than the primer sequences and therefore have a lower melt temperature than the primers. Accordingly, the melt temperature of the amplicon produced by the primers should also have a higher melt temperature than the probes. Thus, as the mixture is cooled, the re-formation of the double stranded amplicon is expected. As previously stated, however, this is not the case. Probes have been found to preferentially bind the single stranded amplicon members. Moreover, this

preference of probe/single stranded amplicon binding exists even when the primer sequences are added in excess of the probes.

After the probe/single stranded amplicon member hybrids are formed, they are detected. Standard heterogeneous assay formats are suitable for detecting the hybrids using the detection labels and capture labels present on the primers and probes. The hybrids can be bound to a solid phase reagent by virtue of the capture label and detected by virtue of the detection label. In cases where the detection label is directly detectable, the presence of the hybrids on the solid phase can be detected by causing the label to produce a detectable signal, if necessary, and detecting the signal. In cases where the label is not directly detectable, the captured hybrids can be contacted with a conjugate, which generally comprises a binding member attached to a directly detectable label. The conjugate becomes bound to the complexes and the conjugates presence on the complexes can be detected with the directly detectable label. Thus, the presence of the hybrids on the solid phase reagent can be determined. Those skilled in the art will recognize that wash steps may be employed to wash away unhybridized amplicon or probe as well as unbound conjugate.

A test sample is typically anything suspected of containing a target sequence. Test samples can be prepared using methodologies well known in the art such as by obtaining a specimen from an individual and, if necessary, disrupting any cells contained therein to release target nucleic acids. The target sequence is either double stranded or single stranded. In the case where PCR is employed in this method, the ends of the target sequences are usually known. In cases where LCR or a modification thereof is employed in the preferred method, the entire target sequence is usually known. Typically, the target sequence is a nucleic acid sequence such as, for example, RNA or DNA.

Generally, two primers which are complementary to a portion of a target strand and its complement are employed in PCR. For LCR, four probes, two of which are complementary to a target sequence and two of which are similarly complementary to the targets complement, are generally employed. While the amplification primers initiate amplification of the target sequence, in some cases, the detection (or hybridization) probe is not involved in amplification. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for example, peptide nucleic acids which are disclosed in International Patent Application WO 92/20702; morpholino analogs which are described in U.S. Patents Nos 5,185,444, 5,034,506, and 5,142,047; and the like. Depending upon the type of label carried by the probe, the probe is employed to capture or detect the amplicon generated by the amplification reaction. The probe is not involved in amplification of the target sequence and therefore may have to be rendered "non-extendable" in that additional dNTPs cannot be added to the probe. In and of themselves analogs usually are non-extendable and nucleic acid probes can be rendered non-extendable by modifying the 3' end of the probe such that the hydroxyl group is no longer

capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified. U.S. Patent Application Serial No. 07/049,061 filed April 19, 1993 describes modifications which can be used to render a probe non-extendable.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences. Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 20 and 50 nucleotides long, more typically in the range of between 20 and 30 nucleotides long. The typical probe is in the range of between 10 and 25 nucleotides long.

Alternatively, a probe may be involved in the amplifying a target sequence, via a process known as "nested PCR". In nested PCR, the probe has characteristics, which are similar to those of the first and second primers normally used for amplification (such as length, melting temperature etc.), and as such, may itself serve as a primer in an amplification reaction. Generally in nested PCR, a first pair of primers (P<sub>1</sub> and P<sub>2</sub>) are employed to form primary extension products. One of the primary primers (for example, P<sub>1</sub>) may optionally be a capture primer (i.e. linked to a member of a first reactive pair), whereas the other primary primer (P<sub>2</sub>) is not. A secondary extension product is then formed using a probe (P<sub>1</sub>') and a probe (P<sub>2</sub>') which may also have a capture type label (such as a member of a second reactive pair) or a detection label at its 5' end. The probes are complementary to and hybridize at a site on the template near or adjacent the site where the 3' termini of P<sub>1</sub> and P<sub>2</sub> would hybridize if still in solution. Alternatively, a secondary extension product can be formed using the P<sub>1</sub> primer with the probe (P<sub>2</sub>') or the P<sub>2</sub> primer with the probe (P<sub>1</sub>') sometimes referred to as "hemi-nested PCR". Thus, a labeled primer/probe set generates a secondary product which is shorter than the primary extension product. Furthermore, the secondary product may be detected either on the basis of its size or via its labeled ends (by detection methodologies well known to those of ordinary skill in the art). In this process, probe and primers are generally employed in equivalent concentrations.

Various methods for synthesizing primers and probes are well known in the art. Similarly, methods for attaching labels to primers or probes are also well known in the art. For example, it is a matter of routine to synthesize desired nucleic acid primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), Dupont (Wilmington, DE), or Milligen (Bedford, MA). Many methods have been described for labeling oligonucleotides such as

the primers or probes of the present invention. Enzo Biochemical (New York, NY) and CLONTECH (Palo Alto, CA) both have described and commercialized probe labeling techniques. For example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (CLONTECH). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (CLONTECH). The amines can be reacted to various haptens using conventional activation and linking chemistries. In addition, copending applications US. Serial Nos. 625,566, filed December 11, 1990 and 630,908, filed December 20, 1990, teach methods for labeling probes at their 5' and 3' termini, respectively. Publications WO92/10505, published 25 June 1992 and WO 92/11388 published 9 July 1992 teach methods for labeling probes at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong *et al.*, Tet. Letters 29(46):5905-5908 (1988); or J. S. Cohen *et al.*, published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' and 5' ends.

Capture labels are carried by the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood, of course that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

Another method provided by the present invention comprises contacting a test sample with a plurality of polynucleotides wherein at least one polynucleotide is provided herein, hybridizing the test sample with the plurality of polynucleotides and detecting the hybridization complexes. The hybridization complexes are identified and quantified to compile a profile which is indicative of vanilloid receptor expression. Expressed RNA sequences may further be detected by reverse transcription and amplification of the DNA product by procedures well-known in the art, including polymerase chain reaction (PCR).

Drug Screening and Gene Therapy.



The present invention also encompasses the use of gene therapy methods for the introduction of antisense vanilloid receptor gene derived molecules such as polynucleotides or oligonucleotides of the present invention into patients with conditions associated with abnormal expression of polynucleotides related to vanilloid receptor. These molecules, including  
5 antisense RNA and DNA fragments and ribozymes, are designed to inhibit the translation of a vanilloid receptor mRNA, and may be used therapeutically in the treatment of conditions associated with altered or abnormal expression of a vanilloid receptor polynucleotide.

Alternatively, the oligonucleotides described above can be delivered to cells by procedures in the art such that the antisense RNA or DNA may be expressed in vivo to inhibit  
10 production of a vanilloid receptor polypeptide in the manner described above. Antisense constructs to vanilloid receptor polynucleotides, therefore, reverse the action of vanilloid receptor transcripts.

The present invention also provides a method of screening a plurality of compounds for specific binding to a human vanilloid receptor polypeptide, or any fragment thereof, to identify  
15 at least one compound which specifically binds a human vanilloid receptor polypeptide. Such a method comprises the steps of providing at least one compound; combining the vanilloid receptor polypeptide with each compound under suitable conditions for a time sufficient to allow binding; and detecting a vanilloid receptor polypeptide binding to each compound. Such a method permits the identification of vanilloid receptor binding compounds which modulate  
20 (i.e. repress or activate) the activity of a vanilloid receptor.

Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the polypeptide of the present invention, is used to design an  
25 antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription, thereby preventing transcription and the production of vanilloid receptor derived polypeptide. For triple helix, see, for example, Lee *et al.*, Nucl. Acids Res. 6:3073 (1979); Cooney *et al.*, Science 241:456 (1988); and Dervan *et al.*, Science 251:1360 (1991) The antisense RNA  
30 oligonucleotide hybridizes to the mRNA in vivo and either blocks translation of an mRNA molecule into the vanilloid receptor polypeptide or alters the transport or stability of the mRNA. For antisense, see, for example, Okano, J. Neurochem. 56:560 (1991); and "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression", CRC Press, Boca Raton, Fla. (1988). Antisense oligonucleotides act with greater efficacy when modified to contain  
35 artificial internucleotide linkages which render the molecule resistant to nucleolytic cleavage. Such artificial internucleotide linkages include but are not limited to methylphosphonate, phosphorothiolate and phosphoroamidate internucleotide linkages.

The polypeptide or peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids which can express the polypeptide or peptide fragment. Drugs may be screened against such transformed cells in competitive binding assays. For example, the formation of complexes between a polypeptide and the agent being tested can be measured in either viable or fixed cells.

The present invention thus provides methods of screening for drugs or any other agent which, upon binding to a vanilloid receptor, improves a condition or disease state. These methods comprise contacting the drug with a vanilloid receptor polypeptide or fragment thereof and assaying for the presence of a complex between the agent and the polypeptide. In competitive binding assays, a labeled agent, which is known to bind to the receptor, typically is used. After suitable incubation, free (or uncomplexed) agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the test agent or drug to bind to receptor polypeptide.

The present invention also encompasses the use of competitive drug screening assays in which neutralizing antibodies capable of binding receptor polypeptide specifically compete with a test drug for binding to the receptor polypeptide or fragment thereof. In this manner, the antibodies can be used to detect the presence of any polypeptide in the test sample which shares one or more antigenic determinants with a polypeptide provided herein.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to at least one receptor polypeptide disclosed herein. Briefly, large numbers of different small molecule test compounds or peptides are synthesized on a solid phase, such as plastic beads or some other surface. The test compounds are reacted with receptor polypeptide and washed. Test compounds thus bound to the solid phase are detected by methods well known in the art. Purified receptor polypeptide can also be coated directly onto plates for use in the drug screening techniques described herein. In addition, non-neutralizing antibodies can be used to capture the receptor polypeptide and immobilize it on the solid support. See, for example, EP 84/03564, published on September 13, 1984.

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of the small molecules including agonists, antagonists, or inhibitors with which they interact. Such structural analogs can be used to fashion drugs which are more active or stable forms of the drug or which enhance or interfere with the function of the receptor polypeptide in vivo. (See, J. Hodgson, Bio/Technology 9:19-21 (1991)).

For example, in one approach, the three-dimensional structure of a receptor polypeptide, or of a receptor polypeptide-inhibitor complex, is determined by x-ray crystallography, by

computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the receptor polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous polypeptide-like molecules or to identify efficient inhibitors.

Useful examples of rational drug design may include molecules which have improved activity or stability as shown by S. Braxton *et al.*, Biochemistry 31:7796-7801 (1992), or which act as inhibitors, agonists, or antagonists of native peptides as shown by S. B. P. Athauda *et al.*, J Biochem. (Tokyo) 113 (6):742-746 (1993).

It also is possible to isolate a target-specific antibody, selected by an assay as described hereinabove, and then to determine its crystal structure. In principle this approach yields a pharmacophore upon which subsequent drug design can be based. It further is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies ("anti-ids") to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-id is an analog of the original receptor. The anti-id then could be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides then can act as the pharmacophore (that is, a prototype pharmaceutical drug).

A sufficient amount of a recombinant receptor polypeptide of the present invention may be made available to perform analytical studies such as X-ray crystallography. In addition, knowledge of the polypeptide amino acid sequence which are derivable from the nucleic acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

Antibodies specific to a human vanilloid receptor polypeptide may further be used to inhibit its biological action by binding to the receptor polypeptide. In this manner, the antibodies may be used in therapy, for example, to treat disorders involving capsaicin-sensitive ion channels.

Further, such antibodies can detect the presence or absence of a human vanilloid receptor polypeptide and, therefore, are useful as diagnostic markers for the diagnosis of disorders involving vanilloid receptors. The present invention also is directed to antagonists and inhibitors of the receptor polypeptides of the present invention. The antagonists and inhibitors are those which inhibit or eliminate the function of the polypeptide. Thus, for example, an antagonist may bind to a receptor polypeptide of the present invention and inhibit or eliminate its function.

The antagonists and inhibitors may be employed as a composition with a pharmaceutically acceptable carrier, including but not limited to saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof.

5    Recombinant Technology.

          The present invention provides vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the present invention and the production of polypeptides of the present invention by recombinant techniques. Such methods comprise culturing the host cells under conditions suitable for the expression of a human vanilloid receptor polynucleotide and recovering the polypeptide produced therefrom from the cell culture.

          a. Host Cells

          In one embodiment, the present invention provides host cells containing a recombinant construct as described below. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or a prokaryotic cell, such as a bacterial cell. Representative examples of appropriate hosts include bacterial cells, such as E. coli, Bacillus subtilis, Salmonella typhimurium; and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a routine matter of choice; fungal cells, such as yeast; insect cells such as *Drosophila* and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings provided herein.

          Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be a cloning vector or an expression vector. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying a vanilloid receptor gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

          b. Vectors and Expression Systems

          The present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. Such vectors include chromosomal, non-chromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, and viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. In a preferred embodiment, a construct comprises an expression vector (as described below). Large numbers of suitable plasmids and

vectors are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example: (a) Bacterial: pBR322 (ATCC 37017); pGEM1 (Promega Biotec, Madison, WI), pUC, pSPORT1 and pProEx1 (Life Technologies, Gaithersburg, MD); pQE70, pQE60, pQE-9 (Qiagen); pBs, phagescript, psiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5, and pGEX4T (Pharmacia Fine Chemicals, Uppsala, Sweden); and (b) Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene); pSVK3, pBPV, pMSG, pSVL (Pharmacia); pcDNA3.1 (Invitrogen). Other appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook *et al.*, *supra*.

Generally however, any plasmid or vector may be used as long as it is replicable and viable in a host.

In a preferred embodiment, the construct is an expression vector which also comprises regulatory sequences operably linked to the sequence of interest, to direct mRNA synthesis and polypeptide production. Regulatory sequences known to operate in prokaryotic and/or eukaryotic cells include inducible and non-inducible promoters for regulating mRNA transcription, ribosome binding sites for translation initiation, stop codons for translation termination and transcription terminators and/or polyadenylation signals. In addition, an expression vector may include appropriate sequences for amplifying expression (such as a dihydrofolate reductase gene).

Promoter regions may be selected from any desired gene but preferably from one which is highly expressed. Particular named bacterial promoters include lacZ, gpt, lambda P sub R, P sub L and trp. Eukaryotic promoters include cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, LTRs from retroviruses, mouse metallothionein-I, prion protein and neuronal specific enolase (NSE). Selection of the appropriate promoter is well within the level of ordinary skill in the art. In addition, a recombinant expression vector will include an origin of replication and selectable marker (such as a gene conferring resistance to an antibiotic (e.g. neomycin, chloramphenicol or ampicillin) or a reporter gene (e.g. luciferase)) which permit selection of stably transformed or transfected host cells.

In a preferred prokaryotic or yeast expression vector, a heterologous structural sequence (i.e. a polynucleotide of the present invention) is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence will encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Preferred eukaryotic expression vectors will also comprise an origin of replication, a suitable promoter operably linked to a sequence of interest and also any necessary translation enhancing sequence, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Such vectors may also include an enhancer sequence to increase transcription of a gene. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription rate. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

i. Vector construction

The appropriate DNA sequence may be inserted into a vector by a variety of procedures. Generally, site-specific DNA cleavage is performed by treating the DNA with suitable restriction enzymes under conditions which are generally specified by the manufacturer of these commercially available enzymes. Usually, about 1 microgram ( $\mu\text{g}$ ) of plasmid or DNA sequence is cleaved by 1 unit of enzyme in about 20 microliters ( $\mu\text{L}$ ) of buffer solution by incubation at 37°C for 1 to 2 hours. After incubation with the restriction enzyme, protein is removed by phenol/chloroform extraction and the DNA recovered by precipitation with ethanol. The cleaved fragments may be separated using polyacrylamide or agarose gel electrophoresis, according to methods known by the routine practitioner. (See Sambrook *et al.*, *supra*).

Ligations are performed using standard buffer and temperature conditions and with a ligase (such as T4 DNA ligase) and ATP. Sticky end ligations require less ATP and less ligase than blunt end ligations. When vector fragments are used as part of a ligation mixture, the vector fragment often is treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase (CIAP) to remove the 5'-phosphate and thus prevent religation of the vector. Alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation. Ligation mixtures are transformed into suitable cloning hosts such as *E. coli* and successful transformants selected by methods including antibiotic resistance, and then screened for the correct construct.

ii. Transformation/Transfection

Transformation or transfection of an appropriate host with a construct of the invention, such that the host produces recombinant polypeptides, may also be performed in a variety of ways. For example, a construct may be introduced into a host cell by calcium chloride transformation, lithium chloride or calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. These and other methods for transforming/transfecting host

cells are well known to routine practitioners (see L. Davis *et al.*, "Basic Methods in Molecular Biology", 2nd edition, Appleton and Lang, Paramount Publishing, East Norwalk, CT (1994)).

iii. Recovery of Expressed Proteins from Recombinant Host Cells

Following transformation or transfection of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction), and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means (to release intracellular protein) and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents; such methods are well-known to the ordinary artisan. When the expressed protein has been secreted, it can be purified directly from the supernatant of harvested cells.

Vanilloid receptor polypeptide is recovered and purified from the supernatant or crude extract by known methods including ammonium sulfate or ethanol precipitation, acid extraction, affinity chromatography, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. It is preferred to have low concentrations (approximately 0.1-5 mM) of calcium ion present during purification (Price, *et al.*, J. Biol. Chem. 244:917 (1969)). Protein refolding steps can be used, as necessary, in completing configuration of the protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

An alternative method for the production of large amounts of secreted protein involves the transformation of mammalian embryos and the recovery of the recombinant protein from milk produced by transgenic cows, goats, sheep, etc. Polypeptides and closely related molecules may be expressed recombinantly in such a way as to facilitate protein purification. One approach involves expression of a chimeric protein which includes one or more additional polypeptide domains not naturally present on human polypeptides. Such purification-facilitating domains include, but are not limited to, metal-chelating peptides such as histidine-tryptophan domains that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase from Invitrogen (San Diego, CA) between the polypeptide sequence and the purification domain may be useful for recovering the polypeptide.

Immunoassays.

The polypeptides including their fragments or derivatives or analogs thereof of the present invention, or cells expressing them, can be used in a variety of assays, many of which are described herein, for the detection of antibodies to a human vanilloid receptor. They also can be used as an immunogen to produce antibodies. These antibodies can be, for example, polyclonal or monoclonal antibodies, chimeric, single chain and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

For example, antibodies generated against a polypeptide corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal such as a mouse, rabbit, chicken, or goat. A mouse, rabbit or goat is preferred. The antibody so obtained then will bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies that bind the native polypeptide. Such antibodies can then be used to isolate the polypeptide from test samples such as tissue suspected of containing that polypeptide. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique as described by Kohler and Milstein, *Nature* **256**: 495-497 (1975), the trioma technique, the human B-cell hybridoma technique as described by Kozbor *et al.*, *Immun. Today* **4**: 72 (1983), and the EBV-hybridoma technique to produce human monoclonal antibodies as described by Cole, *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc, New York, NY, pp. 77-96 (1985). Techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. See, for example, U.S. Pat. No. 4,946,778, which is incorporated herein by reference.

Various assay formats may utilize the antibodies of the present invention, including "sandwich" immunoassays and probe assays. For example, the monoclonal antibodies or fragment thereof of the present invention can be employed in various assay systems to determine the presence, if any, of a vanilloid receptor derived polypeptide in a test sample. For example, in a first assay format, a polyclonal or monoclonal antibody or fragment thereof, or a combination of these antibodies, which has been coated on a solid phase, is contacted with a test sample, to form a first mixture. This first mixture is incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, or a combination of these antibodies, to which a signal generating compound has been attached, is contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is



incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of a vanilloid receptor derived polypeptide antigen present in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the signal generating compound. The amount of vanilloid receptor derived polypeptide antigen present in the test sample is proportional to the signal generated.

Or, a polyclonal or monoclonal vanilloid receptor derived polypeptide antibody or fragment thereof, or a combination of these antibodies which is bound to a solid support, the test sample and an indicator reagent comprising a monoclonal or polyclonal antibody or fragments thereof, which specifically binds to a vanilloid receptor derived polypeptide antigen, or a combination of these antibodies to which a signal generating compound is attached, are contacted to form a mixture. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence, if any, of a vanilloid receptor derived polypeptide present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating compound. The amount of vanilloid receptor derived polypeptide proteins present in the test sample is proportional to the signal generated.

In another assay format, one or a combination of at least two monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to a vanilloid receptor derived polypeptide protein. For example, vanilloid receptor derived polypeptide proteins such as the recombinant antigens disclosed herein, either alone or in combination, are coated on a solid phase. A test sample suspected of containing antibody to a vanilloid receptor derived polypeptide antigen then is incubated with an indicator reagent comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent bound to the solid phase or the indicator reagent bound to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured.

In yet another detection method, each of the monoclonal or polyclonal antibodies of the present invention can be employed in the detection of vanilloid receptor derived polypeptide antigens in fixed tissue sections, as well as fixed cells by immunohistochemical analysis. Cytochemical analysis wherein these antibodies are labeled directly (with, for example, fluorescein, colloidal gold, horseradish peroxidase, alkaline phosphatase, etc.) or are labeled by using secondary labeled anti-species antibodies (with various labels as exemplified herein) to track the histopathology of disease also are within the scope of the present invention.

In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific vanilloid receptor derived

polypeptide proteins from cell cultures or biological tissues such as to purify recombinant and native vanilloid receptor derived polypeptide antigens and proteins.

The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

5 The monoclonal antibodies or fragments thereof can be provided individually to detect vanilloid receptor derived polypeptide antigens. Combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one vanilloid receptor derived polypeptide antibody of the invention with antibodies to other vanilloid receptor derived polypeptide regions, each having different  
10 binding specificities. Thus, this cocktail can include the monoclonal antibodies which are directed to different antigenic determinants of vanilloid receptor derived polypeptide proteins.

The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to a vanilloid receptor derived polypeptide region or other vanilloid receptor derived polypeptide protein used in the assay. The polyclonal antibody used is  
15 preferably of mammalian origin (such as from human, goat, rabbit or sheep). Most preferably, the polyclonal antibody is rabbit polyclonal anti-vanilloid receptor derived polypeptide antibody. The polyclonal antibodies used in the assays can be used either alone or as a cocktail of polyclonal antibodies.

It is contemplated and within the scope of the present invention that a vanilloid receptor  
20 derived polypeptide may be detectable in assays by use of a recombinant antigen as well as by use of a synthetic peptide or purified peptide, which contains an amino acid sequence of a vanilloid receptor derived polypeptide. It also is within the scope of the present invention that different synthetic, recombinant or purified peptides identifying different epitopes of a vanilloid receptor derived polypeptide can be used in combination in an assay to diagnose, evaluate, or  
25 prognose conditions associated with abnormal vanilloid receptor production. In this case, these peptides can be coated onto one solid phase, or each separate peptide may be coated on separate solid phases, such as microparticles, and then combined to form a mixture of peptides which can be later used in assays. Furthermore, it is contemplated that multiple peptides which define epitopes from different polypeptides may be used in combination to make a diagnosis,  
30 evaluation, or prognosis of abnormal vanilloid receptor production. To accomplish this, peptides coated on solid phases or labeled with detectable labels are allowed to compete with peptides from a patient sample for a limited amount of antibody. A reduction in binding of the synthetic, recombinant, or purified peptides to the antibody (or antibodies) is an indication of the presence of vanilloid receptor -secreted polypeptides in the patient sample where it may not  
35 be expected (for example, in cerebral spinal fluid). Such variations of assay formats are known to those of ordinary skill in the art.

In another assay format, the presence of antibody and/or antigen to vanilloid receptor derived polypeptide can be detected in a simultaneous assay, as follows. A test sample is simultaneously contacted with a capture reagent of a first analyte, wherein said capture reagent comprises a first binding member specific for a first analyte attached to a solid phase and a capture reagent for a second analyte, wherein said capture reagent comprises a first binding member for a second analyte attached to a second solid phase, to thereby form a mixture. This mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second analyte complexes. These so-formed complexes then are contacted with an indicator reagent comprising a member of a binding pair specific for the first analyte labeled with a signal generating compound and an indicator reagent comprising a member of a binding pair specific for the second analyte labeled with a signal generating compound to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte/indicator reagent complexes and capture reagent/second analyte/indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated in connection with the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample. In this assay format, recombinant antigens derived from human expression systems may be utilized as well as monoclonal antibodies produced from the proteins derived from the mammalian expression systems as disclosed herein. Such assay systems are described in greater detail in EP Publication No. 0473065.

In yet other assay formats, the polypeptides disclosed herein may be utilized to detect the presence of anti- vanilloid receptor derived polypeptide in test samples. For example, a test sample is incubated with a solid phase to which at least one recombinant protein has been attached. These are reacted for a time and under conditions sufficient to form antigen/antibody complexes. Following incubation, the antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen. In another assay format, a test sample is contacted with a solid phase to which a recombinant protein produced as described herein is attached and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labeled with an indicator reagent. After incubation for a time and under conditions sufficient for antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free phase as an indication of the presence of a vanilloid receptor derived polypeptide antibody. Other assay formats utilizing the recombinant antigens disclosed herein are contemplated. These include contacting a test sample with a solid phase to which at least one antigen from a first source has been attached, incubating the solid phase and test sample for a time and under conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labeled antigen, which antigen is derived from a second source different from the first

source. For example, a recombinant protein derived from a first source such as E. coli is used as a capture antigen on a solid phase, a test sample is added to the so-prepared solid phase, and a recombinant protein derived from a different source (i.e., non-E. coli) is utilized as a part of an indicator reagent. Likewise, combinations of a recombinant antigen on a solid phase and synthetic peptide in the indicator phase also are possible. Any assay format which utilizes an antigen specific for a vanilloid receptor derived polypeptide from a first source as the capture antigen and an antigen specific for vanilloid receptor derived polypeptide from a different second source are contemplated. Thus, various combinations of recombinant antigens, as well as the use of synthetic peptides, purified proteins, and the like, are within the scope of this invention. Assays such as this and others are described in U.S. Patent No. 5,254,458, which enjoys common ownership and is incorporated herein by reference.

Other embodiments which utilize various other solid phases also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer (described in EP publication 0326100 and EP publication No. 0406473), can be employed according to the present invention to effect a fast solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in EPO Publication No. 0 273,115.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including in automated and semi-automated systems wherein the solid phase comprises a microparticle (magnetic or non-magnetic). Such systems include those described in published EPO applications Nos. EP 0 425 633 and EP 0 424 634, respectively.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily adaptable. In scanning probe microscopy, in particular in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunneling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (analyte specific substance which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test

piece which comprises a solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Also, polyelectrolyte interactions may be used to immobilize a specific binding partner on a surface of a test piece by using techniques and chemistries. The preferred method of attachment is by covalent means. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

While the present invention discloses the preference for the use of solid phases, it is contemplated that the reagents such as antibodies, proteins and peptides of the present invention can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the present invention.

It is contemplated that the reagent employed for the assay can be provided in the form of a test kit with one or more containers such as vials or bottles, with each container containing a separate reagent such as a probe, primer, monoclonal antibody or a cocktail of monoclonal antibodies, or a polypeptide (either recombinant or synthetic) employed in the assay. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, may be included in such test kits. It also is contemplated to provide test kits which have means for collecting test samples comprising accessible body fluids, e.g. blood, urine, saliva, and stool. Such collection means include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Test kits designed for the collection, stabilization, and preservation of test specimens obtained by surgery or needle biopsy are also useful. It is contemplated that all kits may be configured in two components; one component for collection and transport of the specimen, and the other component for the analysis of the specimen. Further, kits for the collection, stabilization, and preservation of test specimens may be configured for use by untrained personnel and may be available in the open

market for use at home with subsequent transportation to a laboratory for analysis of the test sample.

The present invention will now be described by way of examples, which are meant to illustrate, but not to limit, the scope of the present invention. All references to the literature, including patents, patent applications and scientific publications, both hereinafter and above, are incorporated by reference in their entirety.

## EXAMPLES

### Example 1: Isolation of Full Length cDNA Clones of Human Vanilloid receptor (hVR1)

The amino acid sequence for the rat vanilloid receptor (Caterina *et al.*, (1997) *supra*) was used to search the LifeSeq™ human expression database (Incyte Pharmaceuticals, Inc., Palo Alto, CA) for human vanilloid receptor (hVR) sequences. A search performed with the rat sequence (using both BLAST and Smith Waterman algorithms) identified two overlapping ESTs (1427917 and 3460342) highly homologous to the rat sequence. These two ESTs overlapped to form a consensus sequence of 270 nt; 1427917 contained the DNA sequence from position 1-227 while 3460342 contained the DNA sequence from position 32-270. The consensus sequence derived from the two ESTs was compared with the published rat VR1 amino acid sequence (SEQ ID NO:4) using the GCG FRAMEALIGN program (FIG. 1) and showed 89 % identity.

PCR primers were prepared from the consensus sequence of ESTs 1427917 and 3460342 to clone the full length gene by RACE PCR (Frohman, MA (1991) *Methods Enzymology* 218:340-362). Two antisense RACE primers corresponding to nucleotide (nt) positions 59-86 and 120-140 from the aligned ESTs (1755 to 1782 and 1816-1836 of SEQ ID NO:7 respectively) were used to prime a human small intestine cDNA library and PCR products were isolated according to the manufacturer's instructions (Marathon Ready cDNA, CLONTECH). cDNAs were obtained which extended 1 kb upstream of the primers. An additional RACE primer was synthesized from the 5'- region of this PCR product (corresponding to nucleotides 855-883 of SEQ ID NO:7) and used to extend the cDNA clones upstream of the translation initiation codon. The sequences down stream of the EST consensus sequence were determined by DNA sequence analysis of the two Incyte clones. The final nucleic acid consensus sequence termed hVR1, (SEQ ID NO:7) and deduced amino acid sequence, (SEQ ID NO:8) were aligned using the GAP Program (Genetics Computer Group, Version 9, University of Wisconsin) with the rat gene and polypeptide sequences (SEQ ID NOs:2 and 4, respectively) and shown to have a DNA sequence identity of 82% (GAP Program, FIG. 2) and an amino acid sequence identity of 86% (GAP Program, FIG. 3). As shown in

FIG. 3, the amino acid sequence from positions 100-800 of SEQ ID NO:8 is 91 % identical to the rat gene product illustrating the sequence divergence at the amino terminal and carboxy terminal ends of the protein.

PCR primers were designed to amplify the hVR1 coding sequence while maintaining the relatively good Kozak consensus sequence for translation (an "A" at position -3 relative to the translation start site) and to provide flanking restriction enzyme sites for subsequent cloning into an expression vector. The sequence of the primers is shown below:

5' Mlu Cap Rec TTAAACGCGTAGGATGAAGAAATGGAGC (SEQ ID NO:5)

Mlu1 start

3' Sal Cap Rec TATATTGTCGACGTCCTCACTTCTCCCCG (SEQ ID NO:6)

Sal1 stop

The hVR1 open reading frame was amplified by PCR using CLONTECH (Palo Alto, CA) human small intestine cDNA as a template and Pfu polymerase (Stratagene®, La Jolla, CA) to ensure a high fidelity product. The resulting PCR product was approximately 2500 bp in length. The PCR product was digested with Mlu1 and Sal1 and cloned into the pCIneo mammalian expression vector (Promega) at the Mlu1 and Sal1 sites. In addition, the PCR product from the human intestine cDNA was sequenced directly.

DNA sequencing of individual clones following transfection of the ligated PCR insert and the vector revealed three forms of the hVR1 gene product. Clone hVR1-1 was identical to SEQ ID NO:7 while hVR1-5 and hVR1-13 each contained two nucleotide substitutions which resulted in amino acid changes in the translation product. Clone hVR1-5 contained both a C to G substitution at position 1144 (designated C1144G) and a C to T substitution at position 1605 (C1605T) in SEQ ID NO:7. Clone hVR1-13 contained both a G1325T substitution and an A1952G substitution. The direct sequencing of the PCR product consistently identified two peaks at positions 1144, 1605 and 1952 (see FIG. 4) suggesting that these variations represent polymorphic gene products. In contrast, only a single peak was found at positions 1325 suggesting that these substitutions were likely to be cloning artifacts. The artifact change at position 1325 for clone hVR1-13 was corrected by site specific mutagenesis using the QuickChange™ Site-Directed Mutagenesis Kit following the protocols of the manufacturer (Stratagene®) and resulted in clones hVR1-13.1.

The polymorphic changes also resulted in amino acid changes in the hVR1 protein. Specifically, the polymorphic variations at nucleotide positions 1144 and 1605 in clone hVR1-5 resulted two amino acid substitutions at position 315 (a methionine rather than an isoleucine) and 469 (an isoleucine rather than a threonine) of SEQ ID NO:8 whereas in clone hVR1-13.1,

the variation at nucleotide position 1952 resulted in a protein containing a valine in place of isoleucine at amino acid position 586 of SEQ ID NO:8.

#### Example 2: Additional Vanilloid Receptor Homologs.

5 The Incyte and dbEST data bases were further searched using Blast and Smith-Waterman algorithms for homologies using the rat and human VR1 amino acid sequences. An additional Incyte EST (1682513) was found with homology in the proposed poor-loop region of rVR1 (Caterina et al., 1997, *supra*). Sequencing clone 1682513 and subsequent RACE  
10 cloning from kidney cDNA libraries identified a 3599 cDNA sequence (SEQ ID NO:1) containing an open reading frame (position 435-3050) coding for an 871 amino acid protein (SEQ ID NO:3) which we termed hVR3.

An alignment of hVR3 with hVR1, rVR1 and hVRL1 (Caterina et al, Nature 398:436-441 1999) using the GCG Pileup program with default parameters shows  
15 significant homology of hVR3 with the other ion channel members (FIG. 3). Particularly significant homology is found in the following structural domains all four sequences (i.e. hVR-1, rVR-1, hVR-2 and hVR-3):

- a) three ankaryn repeat domains, at position 239-270, 294-317 and 370-403 in of FIG. 3
- b) six hydrophobic regions consistent with transmembrane domains, positions 471-493, 519-540, 555-574, 579-597, 621-640 and 703-730
- b) homology in the poor-loop region thought to mediate ion transport, position 671-691

The GAP analysis of hVR1 (bottom sequence) and hVR3 (top sequence) DNA sequences (SEQ ID NOs:7 and 1 respectively) showed 55% sequence identity (FIG. 5) while the GAP analysis of the derived amino acid sequences of hVR1 (bottom sequence) and hVR3 (top sequence), SEQ ID NOs:8 and 3 respectively, showed 46% sequence identity (FIG. 6).

#### Example 3: Quantitative RT-PCR.

Tissue distribution of hVR1 and hVR3 were determined by quantitative PCR (Q-PCR) using the ABI Prism 7700 following the recommendations of the manufacturer (PE-Applied Biosystems, Foster City, CA). The version of Q-PCR we utilized is referred to as TaqMan  
30 PCR.

In TaqMan PCR, a pair of amplification primers that hybridize to the target at sequence defined positions are utilized. In addition, a nucleic acid probe (labeled with a fluorophore and quencher molecule at either end, the TaqMan primer) is utilized that hybridizes to the target at a position between the two primers and preferably adjacent to one of the primers.

35 Importantly, this probe has a melting temperature that is significantly higher (8-10°C) than that of the primers. After the addition of the appropriate reagents (thermostable



polymerase, dNTPs, buffer, Mg<sup>2+</sup>) thermal cycling is begun and as in traditional PCR, the product doubles or nearly doubles each round in exponential fashion. Because the probe has a high melting temperature, during the course of the amplification reaction the polymerase encounters the probe bound tightly to the target at each hybridization/extension phase. The polymerase has a 5'-3' nuclease activity that proceeds to degrade the obstruction and thereby release the fluorophore from the adjacent quencher molecule. After release, the fluorophore can be directly measured via laser excitation at the appropriate wave length. Consequently, the release of the probe fluorophore is directly linked to amplification and quantitative results can be generated by comparison to the amplification of appropriate standards of known concentration.

The amplification primers and nucleic acid probe used in Q-PCR were as follows:

<u>Primer</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>
HVR1-Forward	5'-TGCCTGGAGCTGTTCAAGTTC-3'	SEQ ID NO:9
HVR1-Reverse	5'-TGATGAAGACAGCCTTGAAGTCA-3'	SEQ ID NO:10
HVR1-TaqMan	5'-AGTTCTCAGTGAAGTCCAGGTCGCCCCAT-3'	SEQ ID NO:11
HVR3-Forward	5'-ATTGAGAACCGCCACGAGAT-3'	SEQ ID NO:12
HVR3-Reverse	5'-AGACGGCCCCGAAGTTG-3'	SEQ ID NO:13
HVR3-TaqMan	5'-CCATCAATGAAGTGTGCGGGACA-3'	SEQ ID NO:14

The hVR1 amplification primers corresponded to nucleotide positions 2102-2122 of SEQ ID NO:7 for the forward primer and nucleotide positions 2161-2183 of SEQ ID NO:7 for the reverse primer. The TaqMan primer corresponded to the reverse complement of nucleotide positions 2132-2159 of SEQ ID NO:7. The hVR3 forward, reverse and Taqman primers correspond to positions 1761-1780, 1826-1842 and 1795-1818 of SEQ ID NO:1 respectively. Ten µg total RNA was mixed with 5 µL of 50 ng/µL random hexamers in a final volume of 59 µL water, heated at 70° C for 10 min and placed on ice. The samples were adjusted to 20 mM Tris, pH 8.4, 50 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM DTT, 0.5 mM dNTP and 800 units Superscript® II RT (Gibco BRL) in a 100 µL total volume and incubated at 25° C for 10 min., then 42° C for 50 min followed by 70° C for 10 min. and the samples were placed on ice. The samples were then incubated with 8 units of RNase H at 37° C for 20 min. The cDNA was purified and desalted by filtration on a Chromaspin column (CLONTECH) in TE and the samples quantified by OD<sub>260</sub>. The Q-PCR reactions used 10 ng of cDNA template per 50 µL reaction containing 1X PCR bufferII with 600 nM ROX (PE-Applied Biosystems), 5 mM MgCl<sub>2</sub> and 1.25 units Amplitaq Gold (PE-Applied Biosystems). The samples were incubated at 95° C for 10 min. followed by 40 cycles of 95° C for 15 sec and 60° C for 1 min. Copy number was estimated by a dilution of plasmid DNA containing hVR1 or hVR3 and the cDNA

samples were normalized with Q-PCR reactions using 28S rRNA specific primers. The hVR1 and hVR3 RNA were expressed in relatively low amounts in all of the RNA samples tested (FIG. 7A and B). The hVR1 RNA was most abundant in brain, dorsal root ganglion (DRG), bladder, testes, and kidney while hVR3 was most abundant in kidney and bladder.

#### Example 4: Northern blotting assays.

Alternatively, or in addition to performing quantitative RT-PCR described in Example 2, the well known technique of Northern blotting provides for the detection of messenger RNA and gives a reasonable estimation of its size and steady-state level in a particular tissue (Sambrook *et al.*, *supra*). A Northern blotting assay may be performed as follows: Multiple Tissue Northern Blots are purchased from CLONTECH and probed with a vanilloid receptor cDNA fragment including all or part of SEQ ID NO:1. This fragment is labeled with  $\alpha$ - $^{32}\text{P}$ -dCTP by random priming using a commercial labeling kit (Stratagene®) to a specific activity of  $1.1 \times 10^9$  cpm/ug DNA. The blots (membranes) are prehybridized at 60 °C for 1 hour in Express Hyb solution (supplied with the kit) and hybridized (also in Express Hyb solution) at the same temperature for two hours in the presence of denatured probe at  $2 \times 10^6$  cpm/mL. After washing the blots twice in  $2 \times \text{SSC} + 0.5\% \text{ SDS}$  (20 min each wash), and twice under stringent conditions ( $0.1 \times \text{SSC} + 0.01\% \text{ SDS}$ , 50 °C, 20 min. each wash), the filters are exposed to a phosphorimager screen.

#### Example 5: Ribonuclease Protection Assay

Alternatively, instead of or in addition to performing a Northern blot as described in Example 3, a ribonuclease protection assay may be performed as follows:

A. Labeling of Complementary RNA (cRNA) Hybridization Probes. Labeled sense and antisense riboprobes are transcribed from the EST sequence, which contains an RNA polymerase promoter such as SP6 or T7. The sequence may be from a vector containing the appropriate EST insert or from a PCR-generated product of the insert using PCR primers which incorporate an RNA polymerase promoter sequence. The transcripts are prepared in a 20  $\mu\text{L}$  reaction volume containing 1  $\mu\text{g}$  of DNA template, 2  $\mu\text{L}$  of 100 mM dithiothreitol, 0.8  $\mu\text{L}$  of RNasin (10-40U), 500  $\mu\text{M}$  each of ATP, CTP, GTP, 5  $\mu\text{L}$  ( $\alpha$ - $^{32}\text{P}$ ) UTP or 100-500  $\mu\text{M}$  biotinylated UTP, and 1  $\mu\text{L}$  of RNA polymerase in transcription buffer (40 mM Tris-HCl, pH 7.5, 6 mM  $\text{MgCl}_2$ , 2 mM spermidine HCl, 5 mM NaCl). Following incubation at 37°C for one hour, the transcripts are treated with DNase I (15 U) for an additional 30 min to digest the template. The probes then are isolated by spin columns, salt precipitation or electrophoresis

techniques which are well-known in the art. Finally, the probes are dissolved in lysis buffer (5 M guanidine thiocyanate, 0.1 M EDTA, pH 7.0).

B. Hybridization of Labeled Probe to Target. Approximately 20 µg of extracted total cellular RNA, prepared as described in Sambrook, *et al. supra*, is placed in 10 µL of lysis buffer and mixed with either (i)  $1 \times 10^5$  cpm of radioactively labeled probe or (ii) 250 pg of non-isotopically labeled probe, each in 2 µL of lysis buffer. The mixture then is incubated at 60°C for 5 min and hybridized overnight at room temperature. See, T. Kaabache *et al.*, *Anal. Biochem.* **232**: 225-230 (1995).

C. RNase Digestion. Hybridizations are terminated by incubation with 380 µL of a solution containing 40 µg/mL RNase A and 625 U/mL RNase T1 in 1 mM EDTA, 300 mM NaCl, 30 mM Tris-HCl pH 7.4 for 45-60 min at room temperature. RNase digestion then is terminated by the addition of 60 µL of proteinase-K (1.7 mg/mL) containing 3.3% SDS, followed by incubation for 30 min at 37°C. The digested mixture then is extracted with phenol:chloroform:isoamyl alcohol to remove protein. The mRNA:cRNA hybrids are precipitated from the aqueous phase by the addition 4 µg yeast tRNA and 800 µL of ethanol, and incubation at -80°C for 30 min. The precipitates are collected by centrifugation.

D. Fragment Analysis. The precipitates are dissolved in 5 µL of denaturing gel loading dye (80% formamide, 10 mM EDTA, pH 8.0, 1 mg/mL xylene cyanol, 1 mg/mL bromophenol blue) and electrophoresed in 6 % polyacrylamide TBE, 8 M urea denaturing gels. The gels are dried under vacuum and autoradiographed. Quantification can be performed by comparing the counts obtained from the test samples to a calibration curve that was generated by utilizing calibrators that are the sense strand. In cases where non-isotopic labels are used, hybrids are transferred from the gels to membranes (nylon or nitrocellulose) by blotting and then analyzed using detection systems that employ streptavidin alkaline phosphatase conjugates and chemiluminescence or chemifluorescence reagents. Again, expression of an mRNA which is detectable by the labeled probe in a particular tissue suggests that vanilloid receptor is produced in that tissue.

#### Example 6: Identification of Additional Members of the Vanilloid Receptor Family.

The Northern blot method described in Example 3 *supra* can detect distinct messages only if they have large differences in sizes (more than 100 to 200 nucleotides); small differences in message size (such as those arising from alternative splicing in the coding region) are not detected by this method. Instead, other strategies are used to detect possible variants of vanilloid receptor message and determine their steady-state levels. Splice variants in the coding region can be detected by RT-PCR using primers designed to give products of small size. Variants in the 3' UTR can also be detected by RT-PCR. In RT-PCR, the forward primer is

chosen in a region of the ORF that is common to all message variants known so far, as close as possible to the stop codon. The reverse primer is an oligo-dT anchored with a dinucleotide for the specificity. Since the first nucleotide of the anchor can be A, C, or G, and the second nucleotide can be either A, C, G, or T, a combination of 12 anchored reverse primers are needed. Each reverse primer is thus used with the unique forward primer, in 12 different reactions. The PCR products are then run in an agarose gel and detected by UV fluorescence after ethidium bromide staining. Because of its high sensitivity and specificity, this method allows the detection of even small size and sequence variations in the 3' UTR. Sequence variations at the 5' end of the mRNA can be found using the RACE-PCR technique with similar sensitivity of detecting variant products.

#### Example 7: Dot Blot/Slot Blot

Dot and slot blot assays are quick methods to evaluate the presence of a specific nucleic acid sequence in a complex mix of nucleic acid.

Up to 20 µg of RNA is mixed in 50 µL of 50% formamide, 7% formaldehyde, 1X SSC, and allowed to incubate 15 min at 68°C and cooled on ice. Then, 100 µL of 20X SSC is added to the RNA mixture and loaded onto a vacuum-manifold apparatus that has a prepared nitrocellulose or nylon membrane. The membrane is soaked in water, 20X SSC for 1 hour, placed on two sheets of 20X SSC prewet Whatman #3 filter paper, and inserted into a slot blot or dot blot vacuum manifold apparatus. The slot blot is analyzed with probes prepared and labeled as in Example 4 *supra*.

Other methods and buffers not specifically detailed for Examples 3-5 are described in Sambrook *et al.*, *supra*.

#### Example 8: In Situ Hybridization

This method is useful to directly detect specific target nucleic acid sequences in cells using detectable nucleic acid hybridization probes.

Tissues are prepared with cross-linking fixatives agents such as paraformaldehyde or glutaraldehyde for maximum cellular RNA retention. See, L. Angerer *et al.*, *Methods in Cell Biol.* **35**: 37-71 (1991). Briefly, the tissue is placed in greater than 5 volumes of 1% glutaraldehyde in 50 mM sodium phosphate, pH 7.5 at 4°C for 30 min. The solution is changed with fresh solution for a further 30 min fixing. The fixing solution should have an osmolality of approximately 0.375% NaCl. The tissue is washed once in isotonic NaCl to remove the phosphate.

The fixed tissues then are embedded in paraffin, as follows. The tissue is dehydrated through a series of ethanol concentrations for 15 min each: 50% twice, 70% twice, 85%, 90% and 100% twice. The tissue next is soaked in two changes of xylene for 20 min each at room

temperature; then it is soaked in two changes of 1 xylene:1 paraffin for 20 min each at 60°C; and then it is soaked in three final changes in paraffin for 15 min each.

The tissue next is cut in 5 µm sections using a standard microtome and placed on a slide previously treated with the tissue adhesive 3-aminopropyltriethoxysilane.

5 Paraffin is removed from the tissue by two 10 min xylene soaks and rehydrated in a series of ethanol concentrations; 99% twice, 95%, 85%, 70%, 50%, 30% and distilled water twice. The sections are pre-treated with 0.2 M HCl for 10 min and permeabilized with 2 µg/mL Proteinase-K at 37°C for 15 min.

10 Labeled riboprobes transcribed from the pSPORT1 plasmid containing fragments of vanilloid receptor cDNA are hybridized to the prepared tissue sections and hybridized overnight at 56°C in 3X standard saline extract and 50% formamide. Excess probe is removed by washing in 2X standard saline citrate and 50% formamide followed by digestion with 100 µg/mL RNase A at 37°C for 30 min. Fluorescence probe is visualized by illumination with UV light under a microscope. Fluorescence in the cytoplasm is indicative of mRNA production.

15 Fluorescence in the nucleus detects the presence of genomic material. Alternatively, the sections can be visualized by autoradiography.

#### Example 9: Bacterial Expression and Purification of Human Vanilloid Receptor

##### A. Construction of Expression Vectors containing DNA Fragments Encoding hVR3:

20 DNA fragments encoding hVR3 (containing all or part of SEQ ID NO:1) are generated by PCR for introduction into a prokaryotic expression vector such as pProExI, (Life Technologies, Gaithersburg, MD) using hVR-3 as template DNA. The primers are designed to allow the inframe insertion of the vanilloid receptor fragment with the prokaryotic translation initiation and His tagged regions. After amplification, the PCR products are digested with appropriate  
25 restriction enzymes, and ligated into pProExI, (previously digested with the same restriction enzymes) using standard ligation techniques (see J. Sambrook, *et al. supra*). *E. coli* DH5α cells are then transformed with the ligation mixtures and selected on medium containing ampicillin. Plasmid DNAs are prepared from individual clones and subjected to restriction enzyme analysis to confirm that the hVR3 inserts are in the proper orientation.

30 B. Purification of His-tagged hVR3: In the pProExI expression system, a desired protein is produced with a tag of six histidine residues fused upstream of the protein. Accordingly, the pProExI vectors containing the cloned hVR3 genes or gene fragments thereof are expected to produce fusion proteins of his-tagged hVR3 which could be purified by affinity chromatography to a nickel-conjugated resin. To produce the fusion proteins for purification,  
35 recombinant bacteria are grown overnight in Luria broth containing 50 µg/mL ampicillin (LB + amp) on a rotary shaker at 225 rpm, at 37°C and used to inoculate fresh LB + amp (300 mL) at

a 1:10 dilution. The fresh cultures are incubated, with shaking at 225 rpm, at 37°C for 1 hour, induced with isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG, 1 mM) and re-incubated for an additional 3 hours. Cultures are then centrifuged at 5,000 g to pellet the bacteria. Pellets are resuspended in 10 mL of lysis buffer (50 mM sodium phosphate (pH 8.0), 0.3 M NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.2 mM benzamidine) containing 1% TRITON-X100 at 4°C and sonicated on ice until greater than 90% of the cells are lysed (as determined by OD<sub>590</sub>). After sonication, cell debris and unlysed cells are removed by centrifugation at 10,000 g for 10 minutes at 4°C. The resulting supernatant is loaded onto a 3 mL bed volume nickel-nitro-triacetic acid column (Ni-NTA, QIAGEN, Chatsworth, CA) pre-equilibrated with 10 bed volumes of lysis buffer containing 0.1% TRITON-X100. The column is sequentially washed with 10 bed volumes of wash buffer (50 mM sodium phosphate, pH 6.0, 0.3 M NaCl, and 0.1% TRITON-X100) and 10 bed volumes of 50 mM imidazole in the same buffer (to remove non-specifically bound proteins). The his-tagged vanilloid receptor fusion proteins are eluted from the column with a total of 5 bed volumes of wash buffer containing 0.2 M imidazole and collected as 2 mL fractions. The purity of each eluted fusion protein is assessed after SDS-PAGE on a 13.5% gel stained with Coomassie blue. The protein concentration is determined by absorbance at 280nm after determining the relative extinction coefficients for each of the recombinant fusion proteins or by using the bicinchoninic acid procedure (BCA Protein Assay, Pierce).

#### Example 10: Expression of hVR3 in Eukaryotic Cells

Expression of hVR3 in mammalian cells was achieved by lipofection using the Lipofectamine Plus Reagent (Gibco BRL). HEK 293 cells ( $5 \times 10^6$ ) were plated into 10 cm culture dishes (Falcon 1005) in antibiotic free media (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM Glutamine and 10% fetal bovine serum (FBS), Gibco BRL, Gaithersburg, MD) in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C and grown overnight to approximately 70-80% confluence. Following media replacement (8 mL of the antibiotic free medium), the cells were incubated with a mixture of DNA and Lipofectamine PLUS Reagent (17  $\mu$ g DNA, 147  $\mu$ L PLUS Reagent, 42  $\mu$ L lipofectamine) diluted into 2.1 mLs of serum-free media and incubated for three-four hours. An additional 8 mLs of antibiotic free medium was then added and the cells were incubated overnight. For transient assays, the cells were replated into 96 well plates at  $1 \times 10^5$  cells /well and channel activity was measured the following day (see example 10). For stable cell lines, the neomycin-resistant cells were selected with 800  $\mu$ g/mL Geneticin (Gibco BRL) and the media was replaced frequently to remove dead cells and debris. Individual colonies were obtained by clonal selection, a technique well known in the art.

### Example 11: Functional Analysis of hVR3 in Eukaryotic Cells

A. Functional characterization by FLIPR analysis: A fluorescent imaging plate reader (FLIPR, Molecular Devices, Sunnyvale CA) is used to assay changes in intracellular calcium in cells pre-loaded with the calcium sensitive dye Fluo-3 AM (Molecular Probes, Eugene, Oregon). Either transiently transfected HEK 293 cells or stably transfected subclones of these cells are grown to confluence in black-walled 96-well tissue culture plates in complete medium (antibiotic free medium described in Example 9 + 1% antibiotic/antimycotic (Gibco BRL)+ 200 µg/mL Geneticin). A Fluo-3 AM solution is prepared by dispersing 40 µL of a 1 mg/mL stock Fluo-3/DMSO solution into 10 mL Dulbecco's phosphate-buffered saline (D-PBS). The growth medium is replaced with the Fluo-3 solution and the cells are incubated in the dark at room temperature for 1 hr. The cells are washed gently 3 times with D-PBS using a Denley Cellwash instrument resulting in a final volume of 100 µL D-PBS per well.

Three different plates are loaded into the FLIPR for the assay: (1) the washed HEK 293 cell plate; (2) a plate containing D-PBS or vanilloid receptor antagonist at 4 times the desired concentration; and (3) a plate containing D-PBS or vanilloid receptor agonists at 4 times the desired concentration. The cells are assayed in FLIPR as follows. All pipetting steps are performed by the FLIPR's built-in pipetting armature: 50 µL from the antagonist/D-PBS plate is added to the cell plate 10 seconds after the start of the analysis and incubated for 5 min followed by the addition of 50 µL from the agonist plate and incubation for an additional 10 min. The FLIPR instrument collected fluorescence data throughout the course of the analysis.

B. Functional characterization using *Xenopus* oocytes expression: *Xenopus* oocytes are used for expression of hVR3 and measuring electrophysiological responses essentially as described by Briggs et al. (Neuropharmacology. 1995 Jun;34(6):583-590 [1995]). Female *Xenopus laevis* frogs are obtained from Nasco (Fort Atkinson, WI) and are maintained and treated using standard protocols approved by Abbott's Institutional Animal Care and Use Committee. Frogs are anesthetized with tricaine (0.28%) and sacrificed by decapitation and pithing. Ovaries are removed and placed in low-Ca<sup>2+</sup> Barth's solution (87.5 mM NaCl, 2.5 mM KCl, 1 mM MgCl<sub>2</sub>, and 10 mM Na-HEPES buffer, final pH 7.55). Sections of the ovaries can be maintained at 4°C for up to three weeks for additional oocyte preparations.

For each oocyte isolation, several ovary lobes are taken, opened using blunt dissection, rinsed in low-Ca<sup>2+</sup> Barth's solution, and incubated in collagenase (Type 1A, Sigma Chemical Co., St. Louis, MO; 2 mg/mL in low-Ca<sup>2+</sup> Barth's solution) for 1-2 hours at room temperature. Defolliculation is completed manually. The isolated oocytes are maintained at 17-18°C in normal Barth's solution (90 mM NaCl, 1 mM KCl, 0.66 mM NaNO<sub>3</sub>, 2.4 mM NaHCO<sub>3</sub>, 0.74 mM CaCl<sub>2</sub>, 0.82 mM MgCl<sub>2</sub>, 2.5 mM sodium-pyruvate, 10 mM Na-HEPES buffer (final pH 7.55), 100 U/ml penicillin and 100 µg/ml streptomycin). Oocytes are injected with 50 ng (50

nL of 1 µg/uL) hVR3 RNA within 24 hours of their preparation, and are used within 2-7 days after injection.

Recordings are made using two-electrode voltage clamp in a virtual-ground configuration (Geneclamp 500 amplifier, Axon Instruments, Foster City, CA). Electrodes are made from borosilicate glass (1.5 mM o.d., 1.17 mm i.d.) and are filled with 120 mM KCl. The impedance of the current-passing electrode is determined. Voltage as well as current are recorded to monitor the quality of the voltage clamp and voltage losses are kept below 2 mV.

Experiments are performed in OR2 solution containing 90 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, and 5 mM Na-HEPES buffer, final pH 7.4. The recording chamber (RC5/18, Warner Instruments, Hamden, CT) is perfused at 3 mL/min. Capsaicin is applied for a precisely controlled time using a push/pull applicator positioned to within 300-400 µm from the oocyte and a solenoid valve controlled by the data acquisition system (Digidata 1200 A/D board and pClamp 6 software; Axon Instruments). Antagonists (capsazepine) are superfused in the bathing solution for > 3 minutes prior to testing agonist (capsaicin) in the presence of antagonist. Both antagonist and agonist are present in the ligand applicator so that the concentration of antagonist remains constant during agonist application.

C. Functional characterization using whole cell patch clamping of transfected mammalian cells: HEK-293 cells stably transfected with hVR3 are maintained in the same culture medium as used for FLIPR measurements, but are plated onto glass coverslips in 24-well culture dishes and are used at low cell-density so that recordings so that recordings can be made from individual cells. Whole-cell patch-clamp recordings are made using standard techniques (Axopatch 200B amplifier, Digidata 1200 A/D board and pClamp 6 software; Axon Instruments). Electrodes are made from Corning 7052 glass (1.65 mm o.d., 1.1 mm i.d.; Warner Instrument Corp., Hamden, CT) and have resistances of 1-4 MΩ. The external (bath) solution contains 145 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM dextrose, and 10 mM Na-HEPES buffer (final pH 7.4, 310 mOsm). The internal (pipette) solution contains 130 mM K-aspartate, 10 mM KCl, 10 mM K-BAPTA (Ca<sup>2+</sup> chelator), 2 mM Mg-ATP, and 10 mM K-HEPES buffer (final pH 7.3, 280 mOsm).

Agonists and antagonists are applied to the recorded cell using a DAD-12 computer-controlled micro-superfusion system (ALA Scientific, Westbury, NY). Responses typically are recorded at a holding potential of -60 mV and are normalized to the response to 1 µM capsaicin as a standard to correct for variance of receptor expression from cell to cell. A protocol is devised wherein a compound can be tested in the midst of the standard 1 µM capsaicin application. After applying capsaicin for 20 seconds to determine the standard response, the micro-superfusion is switched immediately to the test compound (e.g., different concentration of capsaicin, or different agonist, or antagonist in the presence of capsaicin) for 20 seconds,



followed immediately by 1  $\mu$ M capsaicin again for 20 seconds to test reversibility of the test compound, and finally back to normal bath solution.

Example 12: Production of Synthetic Peptides of human vanilloid receptor

5 Synthetic peptide sequences (15-25 amino acids) are selected from the hVR3 sequence (SEQ ID NO:8). Peptides are synthesized on an ABI Peptide Synthesizer (available from Applied Biosystems, Foster City, CA), Model 431A, using standard reagents and conditions known in the art for solid phase peptide synthesis (see for example, Stewart, J.M., and Young, D.J., *Solid Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, 1963). Briefly, a  
10 peptide sequence is generated on a resin (such as chloromethyl-polystyrene-divinylbenzene) by the sequential coupling of one or more amino acids or suitably protected amino acids to a growing peptide chain. Cleavage of the peptide from the resin and final deprotection of the peptide are achieved by adding the resin to 20 mL trifluoroacetic acid (TFA), 0.3 mL water, 0.2 mL ethanedithiol, 0.2 mL thioanisole and 100 mg phenol, and stirring at room temperature for  
15 1.5 hours. The resin then is filtered by suction and the peptide obtained by precipitation of the TFA solution with ether, followed by filtration. Each peptide is purified via reverse-phase preparative HPLC using a water/acetonitrile/0.1% TFA gradient and lyophilized. The product is confirmed by mass spectrometry.

20 Example 13: Production of Polyclonal Antibodies to human vanilloid receptor.

A. Preparation of Immunizing Antigens: Purified synthetic peptides are prepared as described in Example 10. To generate antigens for immunization, the purified peptides are conjugated to Keyhole Limpet Hemocyanin (KLH) and bovine serum albumin (BSA) using an Imject Activated Immunogen Conjugation Kit (Pierce, Rockford, Il) in accordance with the  
25 manufacturer's instructions.

B. Immunization Protocol: Polyclonal antisera are generated using the protocol of the Berkeley Antibody Company (Berkeley, CA). Before receiving the first immunization, a sample of preimmune blood (5 mL) is drawn from each of at least 2 rabbits. Afterward, each rabbit is injected subcutaneously with an aliquot of KLH-conjugated peptide (200-500  $\mu$ g) in  
30 Complete Freund's Adjuvant. After 21 days, the immune response is boosted with a second subcutaneous injection of KLH-conjugated peptide (100-250  $\mu$ g) in Incomplete Freund's Adjuvant. Blood (50 mL) is collected on day 31 and serum tested for reactivity to BSA-coupled peptide using an enzyme linked immunoadsorbant assay (ELISA). Subsequent boosts with KLH-conjugated peptide are given on days 42, 63 and 84 (post injection #1) and  
35 production bleeds (50 mL) drawn on days 52, 73 and 94 for testing by ELISA in the manner described. Serum is then stored at -20°C until further use.

Example 14: Inhibition of endogenous vanilloid receptor expression.

Antisense RNA or DNA is a strategy currently widely used to reduce or completely block the endogenous synthesis of proteins . The antisense molecule can be an oligonucleotide targeted to a particular region of the endogenous message, or can be transcribed from an expression vector in which the cDNA for the target gene is ligated in the antisense orientation. In the case of vanilloid receptor, multiple antisense molecules (20-30 nt) are made spanning the complete mRNA and inhibition is measured by the reduction in steady state vanilloid receptor RNA levels in transfected cells using quantitative RT-PCR (Example 2). The oligonucleotides are ranked by their ability to inhibit expression and the best are used in further experiments.

We claim:

1. An isolated or purified polynucleotide derived from human vanilloid receptor, wherein said polynucleotide has at least 83% identity with SEQ ID NO:1, and complements thereof.

2. The polynucleotide of Claim 1 having the nucleotide sequence of SEQ ID NO:1.

3. The polynucleotide of Claim 1 wherein said polynucleotide has a sequence of from about nucleotide position 435 to about nucleotide position 3050 of SEQ ID NO:1.

4. The polynucleotide of Claim 1 wherein said nucleotide sequence selectively hybridizes to SEQ ID NO:1.

5. The polynucleotide of Claim 1 wherein said polynucleotide has an overall length of about 20 to about 50 nucleotides.

6. The polynucleotide of Claim 1 wherein said polynucleotide has an overall length of about 20 to about 25 nucleotides.

7. The polynucleotide of Claim 1, wherein said polynucleotide is produced by recombinant techniques.

8. The polynucleotide of Claim 1, wherein said polynucleotide is produced by synthetic techniques.

9. The polynucleotide of Claim 1 which encodes SEQ ID NO:3.

10. The polynucleotide of Claim 1, wherein said polynucleotide comprises a sequence encoding at least one human vanilloid receptor epitope.

11. The polynucleotide of Claim 1, wherein said polynucleotide is attached to a solid phase.

12. The polynucleotide of Claim 10, wherein said solid phase comprises an array of polynucleotide molecules attached thereto.

13. A recombinant expression system comprising a nucleic acid sequence that includes an open reading frame derived from a human vanilloid receptor polynucleotide, wherein said open reading frame is operably linked to a control sequence compatible with a desired host, and said nucleic acid sequence has at least 83% identity to SEQ ID NO:1, and fragments and complements thereof.

14. The recombinant expression vector of Claim 13 selected from the group consisting of pProEx1 and pCIneo.

15. A host cell transformed with the recombinant expression system of Claim 13.

16. The host cell of Claim 15 wherein said host cell is a eukaryotic cell.

17. A human vanilloid receptor polypeptide encoded by a nucleic acid sequence having at least 83% identity to SEQ ID NO:1.

18. The polypeptide of Claim 17 having SEQ ID NO:3.

19. The polypeptide of Claim 17 produced by recombinant techniques.

20. The polypeptide of Claim 17 produced by synthetic techniques.

21. A method for producing a polypeptide containing at least one human vanilloid receptor epitope comprising incubating host cells transformed with an expression vector wherein said expression vector comprises a nucleotide sequence having at least 83% identity to SEQ ID NO:1.

22. The method of Claim 21 wherein said nucleotide sequence which encodes a human vanilloid receptor has the sequence of SEQ ID NO:1.

23. The method of Claim 21 wherein said nucleotide sequence which encodes a human vanilloid receptor has the sequence of SEQ ID NO:1 from about nucleotide position 435 to about nucleotide position 3050.

24. The method of Claim 21 wherein said nucleotide sequence encodes a human vanilloid receptor having the sequence SEQ ID NO:3.

25. A method for identifying compounds that modulate vanilloid receptor activity, comprising:

- (a) providing a host cell that expresses said vanilloid receptor polypeptide;
- (b) mixing a test compound with said cell; and
- 5 (c) measuring either
  - (i) the effect of the test compound on the cell expressing the receptor, or
  - (ii) the binding of the test compound to the cell or to the receptor.

10 26. The method of claim 25, wherein said host cell is a eukaryotic or procaryotic cell.

27. The method of claim 25, wherein said measurement of step (c) (ii) is performed by measuring a signal generated by a signal-generating compound.

15 28. The method of claim 25, wherein the measurement of step (c) (ii) is performed by measuring a signal generated by a radiolabeled ion, a fluorophore or an electrical current.

20 29. The method of claim 25, further comprising the step of pre-loading the host cell with a fluorescent dye prior to step (b).

30 30. The method of claim 29, wherein the measurement of step (c) (i) is performed by measuring a signal generated by the fluorescent dye.

25 31. A method for identifying a cytoprotective compound, comprising:

- 25 (a) providing a cell that expresses a vanilloid receptor polypeptide or fragment thereof;
- (b) combining a test compound with the cell; and
- (c) monitoring the cell or cellular function for an indication of cytotoxicity.

30 32. The method of Claim 31 wherein said cell is either a procaryotic or eukaryotic cell.

35 33. The method of Claim 31, wherein said cell comprises an expression vector comprising the polynucleotide having at least 83% to SEQ ID NO:1 which is operably linked to control sequences that direct the transcription of the polynucleotide whereby the polynucleotide is expressed in a host cell.

34. The method of claim 31, wherein at least one of the control sequences comprises an inducible promotor.

5 35. The method of claim 34, wherein said cell is maintained in the presence of a substance such as to minimize or block a cytotoxic effect on said cell.

10 36. A method of treating an individual having a condition associated with vanilloid receptor modulation, comprising administering to said individual an effective amount of a compound that controls the gene expression of vanilloid receptor, in a pharmaceutically acceptable excipient.

37. A monoclonal antibody which specifically binds to human vanilloid receptor having amino acid sequence SEQ ID NO:3 or fragments thereof.

15 38. A polyclonal antibody which specifically binds to human vanilloid receptor having amino acid sequence SEQ ID NO:3 or fragments thereof.

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1 CGGCCGTCGATGAAGACCCTGTTTGTGGACAGCTAcAGTGAGATGCTTTTCTTTCTGCAG 60  
|||||||:::|||||...|||||||...|||||||...|||  
500 ArgProSerLeuLysSerLeuPheValAspSerTyrSerGluIleLeuPhePheValGln 519

61 TCACTGTTCATGCTGGCCACCGTGGTGTACTTCAGCCACCTCAAGGAGTATGTGGCT 120  
|||||||...|||||||...|||||||...|||  
520 SerLeuPheMetLeuValSerValValLeuTyrPheSerGlnArgLysGluTyrValAla 539

121 TCCATGGTATTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTC 180  
|||||||:::|||||||...|||||||...|||  
540 SerMetValPheSerLeuAlaMetGlyTrpThrAsnMetLeuTyrTyrThrArgGlyPhe 559

181 CAGCAGATGGGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGT 240  
|||||||...|||||||...|||||||...|||  
560 GlnGlnMetGlyIleTyrAlaValMetIleGluLysMetIleLeuArgAspLeuCysArg 579

241 TTCATGTTTGTCTACATCGTCTTCTTGTTT 270  
|||||||:::|||||||...|||  
580 PheMetPheValTyrLeuValPheLeuPhe 589

FIG. 1

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163 GCAGGTTGCACACTGGGCCACAGAGGATCCAGCAAGGATGAAGAAATGGA 212  
44 GCTGGTTGCAAATTGGGCCACAGAGGATCTGGAAAGGATGGAACAACGGG 93  
213 GCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGC 262  
94 CTAGCTTAGACTCAGAGGAGTCTGAGTCCCCACCCAAGAGAACTCCTGC 143  
263 CCAGACCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCC 312  
144 CTGGACCCTCCAGACAGAGACCCTAACTGCAAGCCACCTCCAGTCAAGCC 193  
313 CCAGCTCTCCACGGCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACT 362  
194 CCACATCTTCACTACCAGGAGTCGTACCCGGCTTTTTGGGAAGGGTGACT 243  
363 CGGAGGAGGCTTTCCCGGTGGATTGCCCTCACGAGGAAGGTGAGCTGGAC 412  
244 CGGAGGAGGCCTCTCCCCTGGACTGCCCTTATGAGGAAGGCGGGCTGGCT 293  
413 TCCTGCCCCGACCATCACAGTCAGCCCTGTTATCACCATCCAGAGGCCAGG 462  
294 TCCTGCCCTATCATCACTGTCAGCTCTGTTCTAACTATCCAGAGGCCTGG 343  
463 AGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTGCGCCGCCA 512  
344 GGATGGACCTGCCAGTGTGAGGCCGTATCCCAGGACTCCGTCTCCGCTG 393  
513 GCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCC 562  
394 G...TGAGAAGCCCCGAGGCTCTATGATCGCAGGAGCATCTTCGATGCT 440  
563 GTTGCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCA 612  
441 GTGGCTCAGAGTAACTGCCAGGAGCTGGAGAGCCTGCTGCCCTTCCTGCA 490  
613 GAAGAGCAAGAAGCACCTCACAGACAACGAGTTCAAAGACCCTGAGACAG 662  
491 GAGGAGCAAGAAGCGCCTGACTGACAGCGAGTTCAAAGACCCAGAGACAG 540  
663 GGAAGACCTGTCTGCTGAAAGCCATGCTCAACCTGCACGACGGACAGAAC 712  
541 GAAAGACCTGTCTGCTAAAAGCCATGCTCAATCTGCACAATGGGCAGAAT 590  
713 ACCACCATCCCCCTGCTCCTGGAGATCGCGCGGCAAACGGACAGCCTGAA 762  
591 GACACCATCGCTCTGCTCCTGGACGTTGCCCGGAAGACAGACAGCCTGAA 640

FIG.2A



763	GGAGCTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGCCAGACAG	812
641	GCAGTTTGTCAATGCCAGCTACACAGACAGCTACTACAAGGGCCAGACAG	690
	·	
813	CACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCTCCTG	862
691	CACTGCACATTGCCATTGAACGGCGGAACATGACGCTGGTGACCCTCTTG	740
	·	
863	GTGGAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAA	912
741	GTGGAGAATGGAGCAGATGTCCAGGCTGCGGCTAACGGGGACTTCTTCAA	790
	·	
913	GAAAACCAAAGGGCGGCCTGGATTCTACTTCGGTGAAGTGGCCCTGTCCC	962
791	GAAAACCAAAGGGAGGCCTGGCTTCTACTTTGGTGAGCTGGCCCTGTCCC	840
	·	
963	TGGCCGCGTGCACCAACCAGCTGGGCATCGTGAAGTTCCTGCTGCAGAAC	1012
841	TGGCTGCGTGCACCAACCAGCTGGCCATTGTGAAGTTCCTGCTGCAGAAC	890
	·	
1013	TCCTGGCAGACGGCCGACATCAGCGCCAGGGACTCGGTGGGCAACACGGT	1062
891	TCCTGGCAGCCTGCAGACATCAGCGCCCGGGACTCAGTGGGCAACACGGT	940
	·	
1063	GCTGCACGCCCTGGTGGAGGTGGCCGACAACACGGCCGACAACACGAAGT	1112
941	GCTTCATGCCCTGGTGGAGGTGGCAGATAACACAGTTGACAACACCAAGT	990
	·	
1113	TTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGGCCAAACTGCAC	1162
991	TCGTGACAAGCATGTACAACGAGATCTTGATCCTGGGGGGCCAAACTCCAC	1040
	·	
1163	CCGACGCTGAAGCTGGAGGAGCTACCAACAAGAAGGGGAATGACGCCGCT	1212
1041	CCCACGCTGAAGCTGGAAGAGATACCAACAGGAAGGGGCTCACGCCACT	1090
	·	
1213	GGCTCTGGCAGCTGGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCC	1262
1091	GGCTCTGGCTGCTAGCAGTGGGAAGATCGGGGTCTTGGCCTACATTCTCC	1140
	·	
1263	AGCGGGAGATCCAGGAGCCCGAGTGCAGGCACCTGTCCAGGAAGTTCACC	1312
1141	AGAGGGAGATCCATGAACCCGAGTGCCGACACCTATCCAGGAAGTTCACC	1190

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1313 GAGTGGGCCTACGGGCCGTGCACTCCTCGCTGTACGACCTGTCCTGCAT 1362  
|||  
1191 GAATGGGCCTATGGGCCAGTGCACTCCTCCCTTTATGACCTGTCCTGCAT 1240

1363 CGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTACAGCAGCA 1412  
|||||  
1241 TGACACCTGTGAAAAGAACTCGGTTCTGGAGGTGATCGCTTACAGCAGCA 1290

1413 GCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGA 1462  
|||  
1291 GTGAGACCCCTAACCGTCATGACATGCTTCTCGTGAACCCCTGAACCGA 1340

1463 CTCCTGCAGGACAAGTGGGACAGATTCTGCAAGCGCATCTTCTACTTCAA 1512  
|||  
1341 CTCCTACAGGACAAGTGGGACAGATTTGTCAAGCGCATCTTCTACTTCAA 1390

1513 CTTCTGCTCTACTGCCTGTACATGATCATCTTCACCATGGCTGCCTACT 1562  
|||  
1391 CTTCTTCGTCTACTGCTTGTATATGATCATCTTCACCGCGGCTGCCTACT 1440

1563 ACAGGCCCGTGGATGGCTTGCCTCCCTTTAAGATGGAAAAAAC...TGGA 1609  
|||  
1441 ATCGGCCTGTGGAAGGCTTGCCCCCTATAAGCTGAAAAACACCGTTGGG 1490

1610 GACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTGTTAGGAGGAGTCTA 1659  
|||  
1491 GACTATTTCCGAGTCACCGGAGAGATCTTGTCTGTGTCAGGAGGAGTCTA 1540

1660 CTTCTTTTTCCGAGGGATTCAATATTTCTGCAGAGGCGGCCGTCGATGA 1709  
|||  
1541 CTTCTTCTTCCGAGGGATTCAATATTTCTGCAGAGGCGACCATCCCTCA 1590

1710 AGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTCTGCAGTCA 1759  
|||  
1591 AGAGTTTGTGTTGTGGACAGCTACAGTGAGATACTTTTCTTGTACAGTCG 1640

1760 CTGTTTCATGCTGGCCACCGTGGTGTGTACTTCAGCCACCTCAAGGAGTA 1809  
|||  
1641 CTGTTTCATGCTGGTGTCTGTGGTACTGTACTTCAGCCAACGCAAGGAGTA 1690

1810 TGTGGCTTCCATGGTATTCTCCCTGGCCTTGGGCTGGACCAACATGCTCT 1859  
|||  
1691 TGTGGCTTCCATGGTGTCTCCCTGGCCATGGGCTGGACCAACATGCTCT 1740

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1860 ACTACACC CGGTTTCCAGCAGATGGGCATCTATGCCGT CATGATTGAG 1909  
||||| ||||| || . ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
1741 ACTATA CCCGAGGATTCCAGCAGATGGGCATCTAT GCTGTCATGATTGAG 1790

1910 AAGATGAT CCTGAGAGACCTGTGCCGTTTCATGTTTG TCTACATCGTCTT 1959  
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
1791 AAGATGAT CCTCAGAGACCTGTGCCGTTTAT GTTCGCTACCTCGTGTT 1840

1960 CTTGTT CGGGTTTTCCACAGCGGTGGTGACGCTGATTGA AGACGGGAAGA 2009  
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
1841 CTTGTTT GGATTTTCCACAGCTGTGGTGACACTGATTG AGGATGGGAAGA 1890

2010 ATGACT CCCTGCCGTCTGAGTCCACGTCGCACAGGTGG CGGGGGCCTGCC 2059  
|| | ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
1891 ATA ACTCTCTGCCTATGGAGTCCACACCACACAAGT GCCGGGGGTCTGCC 1940

2060 TGCAGG CCCCCGATAGCTCCTACAACAGCCTGTACTCC ACCTGCCTGGA 2109  
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
1941 TGCAAG . . . CCAGGTA ACTCTTACAACAGCCTGT ATTCCACATGTCTGGA 1987

2110 GCTGTT CAAGTTCACCATCGGCATGGGCGACCTGGAG TTCACTGAGAACT 2159  
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
1988 GCTGTT CAAGTTCACCATCGGCATGGGCGACCTGGAG TTCACTGAGAACT 2037

2160 ATGACT TCAAGGCTGTCTTCATCATCCTGCTGCTGGC CTATGTAATTCTC 2209  
| | ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
2038 ACGACT TCAAGGCTGTCTTCATCATCCTGTTACTGGC CTATGTGATTCTC 2087

2210 ACCTAC ATCCTCTGCTCAACATGCTCATCGCCCTCAT GGGTGAGACTGT 2259  
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
2088 ACCTAC ATCCTTCTGCTCAACATGCTCATTGCTCTCAT GGGTGAGACCGT 2137

2260 CAACAAG ATCGCACAGGAGAGCAAGAACATCTGGAAG CTGCAGAGAGCCA 2309  
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
2138 CAACAAG ATTGCACAAGAGAGCAAGAACATCTGGAAG CTGCAGAGAGCCA 2187

2310 TCACCAT CCTGGACACGGAGAAGAGCTTCCTTAAGT GCATGAGGAAGGCC 2359  
|| | ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
2188 TCACCAT CCTGGATACAGAGAAGAGCTTCCTGAAGT GCATGAGGAAGGCC 2237

2360 TTC CGCTCAGGCAAGCTGCTGCAGGTGGGGTACACAC CTGATGGCAAGGA 2409  
||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
2238 TTC CGCTCTGGCAAGCTGCTGCAGGTGGGGTTCACT CCTGACGGCAAGGA 2287

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2410 CGACTACCGGTGGTGTTCAGGGTGGACGAGGTGAACTGGACCACCTGGA 2459  
|||||  
2288 TGACTACCGGTGGTGTTCAGGGTGGACGAGGTAACTGGACTACCTGGA 2337  
2460 ACACCAACGTGGGCATCATCAACGAAGACCCGGGCAACTGTGAGGGCGTC 2509  
|||||  
2338 ACACCAATGTGGGTATCATCAACGAGGACCCAGGCAACTGTGAGGGCGTC 2387  
2510 AAGCGCACCTGAGCTTCTCCCTGCGGTCAAGCAGAGTTTCAGGCAGACA 2559  
|||||  
2388 AAGCGCACCTGAGCTTCTCCCTGAGGTCAAGCCGAGTTTCAGGGAGAAA 2437  
2560 CTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCTCGAG 2609  
|||||  
2438 CTGGAAGAACTTTGCCCTGGTCCCCCTTCTGAGGGATGCAAGCACTCGAG 2487  
2610 ATAGGCAGTCTGCTCAGCCCAGGAAGTTTATCTGCGACAGTTTTCAGGG 2659  
|||||  
2488 ATAGACATGCCACCCAGCAGGAAGAAGTTCAACTGAAGCATTATACGGGA 2537  
2660 TCTCTGAAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGG 2709  
|||  
2538 TCCCTTAAGCCAGAGGATGCTGAGGTTTTCAAGGATTCCATGGTCCCAGG 2587  
2710 GGAGAAAGTGA.GGACGTCA CGCAGACAGCACTGTCAACACTGGGCCTTAG 2758  
|||||  
2588 GGAGAAATAATGGACACTATGCAGGGA.....TCAATGCGGGGTCTT.. 2629  
2759 GAGACCCCGTTGCCACGGGGGGCTTGCTGAGGGAA.CACCAGTGCTCTGT 2807  
|||  
2630 .....TGGGTGGTCTGCTTAGGGAACCAGCAGGG..TTGA 2662  
2808 CAGCAGCCTGGCCTGGTCTGTGCCTGCCCA.GCATGTTCCCAAATCTGTG 2856  
|||  
2663 CGTTATCTGGGTCCACTCTGTGCCTGCCTAGGCACATTCCTAGGACTTCG 2712  
2857 CTGGACAAGCTGTGGGAA 2874  
|||  
2713 GCGGGCCTGCTGTGGGAA 2730

FIG.2E

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	1					60
hvr1	-----	-----	-----	-----	MKKW SSTD LGAAAD	PLQKDTCPDP
rvr1	-----	-----	-----	-----	MEQR ASLDSEES	PPQENSCLDP
hvr2	-----	-----	-----	-----		
hvr3	MADSSEGPR	GPGEVAELPG	DESGTPGGEA	FPLSSLANLF	EGEDGSLSPS	PADASRPAGP
Consensus	-----	-----	-----	-----	-----	-----
	61					120
hvr1	LDGDPNSRPP	PAKPQLSTAK	SRTRLFGKGD	SEEAFVDCP	HEEGELD.SC	PTITVSPVIT
rvr1	PDRDPNCKPP	PVKPHIFTTR	SRTRLFGKGD	SEEASPLDCP	YEEGGLA.SC	PIITVSSVLT
hvr2	-----	-----	-----	-----	-----	-----
hvr3	GDGRPNLRMK	FQGAFRKGV	NPIDLLESTL	YESSVVP	KAPMDSLFDY	GTYRHHSS.D
Consensus	-----	-----	-----	-----	-----	-----
	121					180
hvr1	IQRPGDGPTG	ARLLSQDSVA	ASTEKTLRLY	DRRSIFEAVA	QNNCQDLESL	LLFLQSKKH
rvr1	IQRPGDGPAS	VRPSSQDSVS	AG.EKPPRLY	DRRSIFDAVA	QSNQCELESL	LPFLQSKKR
hvr2	GEDRKFAPI	RVNLNYRKGT	GASQDPNRF	DRDRLFNAVS	RGVPEDLAGL	PEYLSKTSKY
hvr3	NKRWRKKIIE	KQPQSPKAPA	PQPPPILKVF	NRPIILFDIVS	RGSTADLDGL	LPFLLTTHKKR
Consensus	-----	-----	-----	-R--F--V-	-----L--L	---L---K-
	181					240
hvr1	LTDNEFKDPE	TGKTCLLKAM	LNLHDGQNTT	IPLLLLEIARQ	TDSLKELVNA	SYTDSYYKGG
rvr1	LTDSEFKDPE	TGKTCLLKAM	LNLHNGQNDT	IALLLDVARK	TDSLKQFVNA	SYTDSYYKGG
hvr2	LTDSEYTEGS	TGKTCLMKAV	LNLKDGVNAC	ILPLLQIDRD	SGNPQPLVNA	QCTDDYYRGH
hvr3	LTDEEFREPS	TGKTCLPKAL	LNLNGRNDT	IPVLLDIAER	TGNMREFINS	PFRDIYYRGQ
Consensus	LTD-E-----	TGKTCL-KA-	LNL--G-N--	I--LL-----	-----N-	---D-YY-G-
	241					300
hvr1	TALHIAIERR	NMALVTLLVE	NGADVQAAAH	GDFFKTKGR	PGFYFGELPL	SLAACTNQLG
rvr1	TALHIAIERR	NMTLVTLLE	NGADVQAAAN	GDFFKTKGR	PGFYFGELPL	SLAACTNQLA
hvr2	SALHIAIEKR	SLOQVKLLVE	NGANVHARAC	GRFFQKGQG.	TCFYFGELPL	SLAACTKQWD
hvr3	TALHIAIERR	CKHYVELLVA	QGADVHAQAR	GRFFQPKDEG	GYFYFGELPL	SLAACTNQPH
Consensus	-ALHIAIE-R	----V-LLV-	-GA-V-A-A-	G-FF-----	--FYFGELPL	SLAACT-Q--
	301					360
hvr1	IVKFLLQNSW	QTADISARDS	VGNTVLHALV	EVADNTADNT	KFVTSMYNEI	LILGAKLHPT
rvr1	IVKFLLQNSW	QPADISARDS	VGNTVLHALV	EVADNTVDNT	KFVTSMYNEI	LILGAKLHPT
hvr2	VVSYLENPH	QPASLOATDS	QGNTVLHALV	MISDNSENI	ALVTSMYDGL	LQAGARLCPT
hvr3	IVNYLTENPH	KKADMRRQDS	RGNTVLHALV	AIADNTRENT	KFVTKMYDLL	LLKCARLFPD
Consensus	-V--L--N--	--A-----DS	-GNTVLHALV	---DN---N-	--VT-MY---	L---A-L-P-
	361					420
hvr1	LKLEELTNKK	GMTPLALAAG	TGKIGVLAYI	LQREIQEPEC	RHL SRKFTEW	AYGPVHSSLY
rvr1	LKLEELITNRK	GLTPLALAAS	SGKIGVLAYI	LQREIHEPEC	RHL SRKFTEW	AYGPVHSSLY
hvr2	VQLEDIRNLQ	DLTPLKLAAK	EGKIEIFRHI	LQREFS..GL	SHL SRKFTEW	CYGPVRVSLY
hvr3	SNLEAVLNND	GLSPLMMAAK	TGKIGIFOHI	IRREVTDEDT	RHL SRKFKDW	AYGPVYSSLY
Consensus	--LE--N--	---PL--AA-	-GKI-----I	-RE-----	-HLSRKF--W	-YGPV--SLY
	421					480
hvr1	DLSCIDTC.E	KNSVLEVIAY	SSSETPNRHD	MLLVEPLNRL	LQDKWDRFVK	RIFYFNFLVY
rvr1	DLSCIDTC.E	KNSVLEVIAY	SSSETPNRHD	MLLVEPLNRL	LQDKWDRFVK	RIFYFNFFVY
hvr2	DLASVDSC.E	ENSVLEIIAF	.HCKSPHRHR	MVVLEPLNKL	LQAKWDLIP	K.FFLNFLCN
hvr3	DLSSLDTCGE	EASVLEILVY	.NSKIENRHE	MLAVEPINEL	LRDKWRKFGA	VSFYINVVSY
Consensus	DL---D-C-E	--SVLE----	-----RH-	M---EP-N-L	L--KW-----	--F--N----

FIG.3A

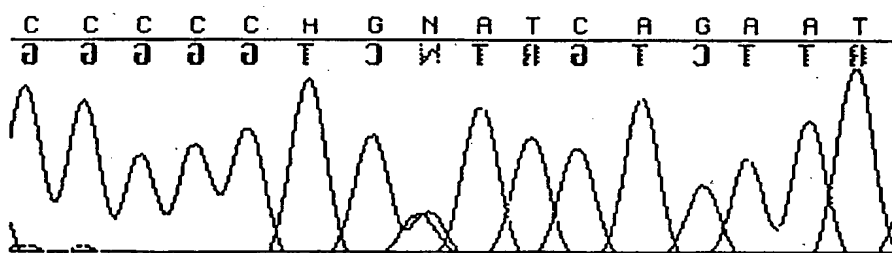
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	481					540
hvr1	CLYMIIFTMA	AYYRP.VDGL	PPFKMEKT.G	DYFRVTGEIL	SVLGGVYFFF	RGIQ.YFLQR
rvr1	CLYMIIFTAA	AYYRP.VEGL	PPYKLKNTVG	DYFRVTGEIL	SVSGGVYFFF	RGIQ.YFLQR
hvr2	LIYMFIFTAV	AYHQPTLKKQ	AAPHLKAEVG	NSMLLTGHIL	ILLGGIYLLV	GQLW.YFWRR
hvr3	LCAMVIFTLT	AYYQP.LEGT	PPYPYRTTV.	DYLRLAGEVI	TLFTGVLFFF	TNIKDLFMKK
Consensus	---M-IFT--	AY--P----	-----	-----G---	----G-----	-----F---
	541					600
hvr1	RPSMKTLFVD	SYSEMLFFLQ	SLFMLATVVL	YFSLKEYVA	SMVFSLALGW	TNMLYYTRGF
rvr1	RPSLKSFLVD	SYSEILFFVQ	SLFMLVSVVL	YFSQRKEYVA	SMVFSLAMGW	TNMLYYTRGF
hvr2	HVFIWISFID	SYFEILFLFQ	ALLTVSVQVL	CFLAIEWYLP	LLVSALVLGW	LNLLYYTRGF
hvr3	CPGVNSLFID	GSFQLLYFIY	SVLVIVSAAL	YLAGIEAYLA	VMVFALVLGW	MNALYFTRGL
Consensus	-----F-D	-----L----	-----L	-----Y--	--V--L--GW	-N-LY-TRG-
	601					660
hvr1	QMGIIYAVMI	EKMILRDLCR	FMFVYIVFLF	GFSTAVVTLI	EDGKNDSL.P.	...SESTSHR
rvr1	QMGIIYAVMI	EKMILRDLCR	FMFVYLVLFL	GFSTAVVTLI	EDGKNNSLP.	...MESTPHK
hvr2	QHTGIYSVMI	QKVILRDLLR	FLLIYLVFLF	GFAVALVSL	QEAWRPEAPT	GPNATESVQP
hvr3	KLTGTYSIMI	QKILFKDLFR	FLLVYLLFMI	GYASALVSL	NPCANMKVCN	EDQTNCTVPT
Consensus	---G-Y--MI	-K----DL-R	F---Y--F--	G---A-V-L-	-----	-----
	661					720
hvr1	WRGPACRPPD	SSYNSLYSTC	LELFKFTIGM	GLEFTENYD	FKAVFIILL	AYVILTYILL
rvr1	CRGSACK.PG	NSYNSLYSTC	LELFKFTIGM	GLEFTENYD	FKAVFIILL	AYVILTYILL
hvr2	MEGQEDEGNG	AQYRGILEAS	LELFKFTIGM	GELAFQEQLH	FRGMVLLLLL	AYVLLTYILL
hvr3	YPSCRDSETF	STF.....L	LDLFKLTIGM	GOLEMLSSTK	YPVVFILLV	TYIILTFVLL
Consensus	-----	-----	L-LFK-TIGM	G-L-----	-----LL-	-Y--LT--LL
	721					780
hvr1	LNMLIALMGE	TVNKIAQESK	NIWKLQRAIT	ILDTEKSFLK	CMRKAFRSGK	LLQVGYTPDG
rvr1	LNMLIALMGE	TVNKIAQESK	NIWKLQRAIT	ILDTEKSFLK	CMRKAFRSGK	LLQVGFTPDG
hvr2	LNMLIALMSE	TVNSVATDSW	SIWKLOKAIS	VLXMENGYWW	C.RKKQRAGV	MLTVGTPKPDG
hvr3	LNMLIALMGE	TVGQVSKESE	HIWKLQWATT	ILDIERSPFV	FLRKAFRSGE	MVTVGKSSDG
Consensus	LNMLIALM-E	TV-----S-	-IWKLQ-A--	-L--E-----	--RK--R-G-	---VG---DG
	781					840
hvr1	KDDYRWCFRV	DEVNWTWNT	NVGIINEDPG	NCEGVKRTLS	FSLRSS....	RVSGRHWKNF
rvr1	KDDYRWCFRV	DEVNWTWNT	NVGIINEDPG	NCEGVKRTLS	FSLRSG....	RVSGRNWKNF
hvr2	SPDERWCFRV	EEVNWASWEQ	TLPTLCEDPS	GA.GVPRTLE	NPVLASPPKE	DEDGASEENY
hvr3	TPDRRWCFRV	DEVNWSHWNQ	NLGIINEDPG	KNE....TYQ	YYGFSHTVGR	LRRDRWSSVV
Consensus	--D-RWCFRV	-EVNW--W--	-----EDP-	-----T--	-----	-----
	841					889
hvr1	ALVPLLREAS	ARDRQSAQPE	EVYLRQFSGS	LKPEDAIEVFK	SPAASGEK*	
rvr1	ALVPLLPDAS	TRDRHATQQE	EVQLKHYTGS	LKPEDAIEVFK	DSMVPGEK~	
hvr2	VPVQLLQSN*	-----	-----	-----	-----	
hvr3	PRVVELNKNS	NPDEVVVPLD	SMGNPRCDGH	QQGYPRKWRT	DDAPL*~	
Consensus	--V--L----	-----	-----	-----	-----	

FIG.3B

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↑ 1144, C &amp; G

A A A A A N T G G A G  
T T T T T T A T T T



A A A A A C T G G A G F  
A A A A A C T G G A G A



↑ 1605, C &amp; T

C T A C A T C G T C T T  
T C T A C A T C G T C T T C



T C T A C A T C G T C T T C  
T C T A C A T C G T C T T C



↑ 1952, A &amp; G

FIG.4

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301 CTGAAATGTTAGGCAGATA<sup>˙</sup>CCCTGTGGGAGCTTTGTTCTGGGATGCTAAGA<sup>˙</sup>ACCGCTTGA<sup>˙</sup> 360  
1 .....GAGGTTCA<sup>˙</sup>GTCT<sup>˙</sup>CGGAAAC<sup>˙</sup>ACTTCAGTTCTAGGGGGCTGGGGGCAGCAGCA<sup>˙</sup> 51  
361 GGATTTAAGC<sup>˙</sup>TTTGCCACTTTGGCTCCGGAGCAAGGGCAGAGGGCTGAGCAGTGCAGACG<sup>˙</sup> 420  
52 AGTTGGAGTTT<sup>˙</sup>TGGGGTACCCTGCTTC....ACAGGGCCCTTGGCAAGGAGGGCAGGTGG<sup>˙</sup> 107  
421 GGCCTGGGGCAGGCATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCGGGGAGGTGG<sup>˙</sup> 480  
108 GGTCTAAGGACAAGCAGTCCTTACTTTGGGAGTCAACCCCGGCGTGGTGGCTGCTGCAGG<sup>˙</sup> 167  
481 CTGAGCTCCC<sup>˙</sup>CGGGGATGAGAGTGGCACC<sup>˙</sup>CCAGGTGGGGAGGCTTTTCTCTCTCCTCCC<sup>˙</sup> 540  
168 TT..GCACACTGGGCCACAGAGGATCCAGCAAGGATGAAGAAATGGAGCAGCACAGACTT<sup>˙</sup> 225  
541 TGGCCAATCTGTTTGAGGGGAGGATGGCTCCCTTTGCGCCTCACCGGCTGATGCCAGTC<sup>˙</sup> 600  
226 GGGGGCAGCTGCGGACCACTCCAAAAGGACACCTGCCCAGACCCCTGGATGGAGACCC<sup>˙</sup> 285  
601 GCCCTGCTGGCCAGGCGATGGGCGACCA<sup>˙</sup>AATCTGCGCATGAAGTTCCAGGGCGCCTTCC<sup>˙</sup> 660  
286 TAACTCCAGGCC.....ACCTCCAGCCAAGCCCCAGCTCTCCACGG<sup>˙</sup> 326  
661 GCAAGGGGGTGCCCAACCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGG<sup>˙</sup> 720  
327 CCAAGAG...CCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGG<sup>˙</sup> 383  
721 TGCCTGGGCCCAAGAAAGC<sup>˙</sup>ACCCATGGACTCACTGTTTGACTACGGCACCTATCGTCACC<sup>˙</sup> 780  
384 ATTGCCCTCACGAGGAAGGTGAGCTGGACTC.TGCCCCGACCATCACAGTCA.....GCC<sup>˙</sup> 437  
781 ACTCCAGTGACAACAAGAGGTGGAGGAAGAAGATCATAGAGAAGCAGCCGCAGAGCCCCA<sup>˙</sup> 840  
438 CTGTTATCACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGG<sup>˙</sup> 497  
841 AAGCCCCTGCCCTCAGCGCCCCCATCCTCAAAGTCTTCAACCGGCCTATCCTCTTTG<sup>˙</sup> 900  
498 ACTCTGTGCGCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTG<sup>˙</sup> 557  
901 ACATCGTGTCCCGGGGCTCCACTGCTGACCTGGACGGGCTGCTCCCATTCTTGCTGACCC<sup>˙</sup> 960  
558 AAGCCGTTGCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGA<sup>˙</sup> 617  
961 ACAAGAAACGCCTAACTGATGAGGAGTTTCGAGAGCCATCTACGGGGAAGACCTGCCTGC<sup>˙</sup> 1020  
618 GCAAGAAGCACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGC<sup>˙</sup> 677  
1021 CCAAGGCCTTGCTGAACCTGAGCAATGGCCGCAACGACACCATCCCTGTGCTGCTGGACA<sup>˙</sup> 1080  
678 TGAAAGCCATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGA<sup>˙</sup> 737

FIG.5A



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1081 TCGCGGAGCGCACCGGCAACATGCGGGAGTTCATTAACTCGCCCTTCCGTGACATCTACT 1140  
738 TCGCGCGGCAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACGGACAGCTACT 797  
1141 ATCGAGGTCAGACAGCCCTGCACATCGCCATTGAGCGTCGCTGCAAACACTACGTGGAAC 1200  
798 ACAAGGGCCAGACAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCC 857  
1201 TTCTCGTGGCCCAGGGAGCTGATGTCCACGCCAGGCCCGTGGGCGCTTCTTCCAGCCCA 1260  
858 TCCTGGTGGAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAA 917  
1261 AGGATGAGGGGGGCTACTTCTACTTTGGGGAGCTGCCCCGTGCTGCTGGCTGCCTGCACCA 1320  
918 CCAAAGGGCGGCCTGGATTCTACTTCGGTGAACTGCCCTGTCCCTGGCCGCGTGACCA 977  
1321 ACCAGCCCCACATTGTCAACTACCTGACGGAGAACCCCCACAAGAAGGCGGACATGCGGC 1380  
978 ACCAGCTGGGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCG 1037  
1381 GCCAGGACTCGCGAGGCAACACAGTGCTGCATGCGCTGGTGGCCATTGCTGACAACACC 1440  
1038 CCAGGGACTCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCCGACAACACGG 1097  
1441 GTGAGAACACCAAGTTTGTACCAAGATGTACGACCTGCTGCTGCTCAAGTGTGCCCGCC 1500  
1098 CCGACAACACGAAGTTTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGCCAAAC 1157  
1501 TCTTCCCCGACAGCAACCTGGAGGCCGTGCTCAACAACGACGGCCTCTCGCCCCTCATGA 1560  
1158 TGCACCCGACGCTGAAGCTGGAGGAGCTACCAACAAGAAGGGAATGACGCCGCTGGCTC 1217  
1561 TGGCTGCCAAGACGGGCAAGATTGGGATCTTTCAGCACATCATCCGGCGGGAGGTGACGG 1620  
1218 TGGCAGCTGGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGG 1277  
1621 ATGAGGACACACGGCACCTGTCCCGCAAGTTCAAGGACTGGGCCTATGGGCCAGTGTATT 1680  
1278 AGCCCGAGTGACAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCCTGCACT 1337  
1681 CCTCGCTTTATGACCTCTCCTCCCTGGACACGTGTGGGGAAGAGGCCTCCGTGCTGGAGA 1740  
1338 CCTCGCTGTACGACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGA 1397  
1741 TCCTGGTGTACAACAGCAAGATTGAGAACCGCCACGAGATGCTGGCTGTGGAGCCCATCA 1800  
1398 TCGCCTACAGCAGCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGA 1457  
1801 ATGAACTGCTGCGGGACAAGTGGCGCAAGTTCGGGGCCGTCTCCTTCTACATCAACGTGG 1860  
1458 ACCGACTCCTGCAGGACAAGTGGGACAGATTGTCGAAGCGCATCTTCTACTTCAACTTCC 1517

FIG.5B

1861 TCTCCTACCTGTGTGCCATGGTCATCTTCACTCTCACCGCCTACTACCAGCCGCTGGAGG 1920  
| | | | | | | | | | | | | | | | | | | | | |  
1518 TGGTCTACTGCCTGTACATGATCATCTTACCATGGCTGCCTACTACAGGCCCGTGATG 1577  
  
1921 GCACACCGCCGTACCCTTAACCGCACCACGGTGGACTACCTGCGGCTGGCTGGCGAGGTC A 1980  
| | | | | | | | | | | | | | | | | | | | | |  
1578 GCTTGCCTCCCTTTAAGATGGAAAAAACTGGAGACTATTTCCGAGTTACTGGAGAGATCC 1637  
  
1981 TTACGCTCTTCACTGGGGTCTGTCTTCTTACCAACAATCAAAGACTTGTTTCATGAAGA 2040  
| | | | | | | | | | | | | | | | | | | | | |  
1638 TGTCTGTGTTAGGAGGAGTCTACTTCTTTTTCCGAGGGAT...TCAGTATTTCTGCAGA 1694  
  
2041 AATGCCCTGGAGTGAATTCTCTTTCATTGATGGCTCCTTCCAGCTGCTCTACTTCATCT 2100  
| | | | | | | | | | | | | | | | | | | | | |  
1695 GGCGGCCGTCGATGAAGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTTCTGC 1754  
  
2101 ACTCTGTCCTGGTGATCGTCTCAGCAGCCCTCTACCTGGCAGGGATCGAGGCCTACCTGG 2160  
| | | | | | | | | | | | | | | | | | | | | |  
1755 AGTCACTGTTTCATGCTGGCCACCGTGGTGTCTGTACTTCAGCCACCTCAAGGAGTATGTGG 1814  
  
2161 CCGTGATGGTCTTTGCCCTGGTCTGGGCTGGATGAATGCCCTTTACTTCACCCGTGGGC 2220  
| | | | | | | | | | | | | | | | | | | | | |  
1815 CTTCCATGGTATTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTT 1874  
  
2221 TGAAGCTGACGGGGACCTATAGCATCATGATCCAGAAGATTCTCTTCAAGGACCTTTTCC 2280  
| | | | | | | | | | | | | | | | | | | | | |  
1875 TCCAGCAGATGGGCATCTATGCCGTCATGATTGAGAAGATGATCCTGAGAGACCTGTGCC 1934  
  
2281 GATTCTGCTCGTCTACTTGCTCTTCATGATCGGCTACGCTTCAGCCCTGGTCTCCCTCC 2340  
| | | | | | | | | | | | | | | | | | | | | |  
1935 GTTTCATGTTTGTCTACATCGTCTTCTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGA 1994  
  
2341 TGAACCCGTGTGCCAACATGAAGGTGTGCAATGAGGACCAGACCAACTGCACAGTGCCCCA 2400  
| | | | | | | | | | | | | | | | | | | | | |  
1995 TTGAAGACGGGAAGAATGACTCCCTGCCGTCTGAGTCCACGTCGCACAGGTGGCGGGGGC 2054  
  
2401 CTTACCCCTCGTGCCGTGACAG.....CGAGACCTTCAGCACCTTCCTCCTGGACCTGT 2454  
| | | | | | | | | | | | | | | | | | | | | |  
2055 CTGCCTGCAGGCCCCCCCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGT 2114

**SUBSTITUTE SHEET (RULE 26)**

2455 TTAAGCTGACCATCGGCATGGGCGACCTGGAGATGCTGAGCAGCACCAAGTACCCCGTGG 2514  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2115 TCAAGTTACCATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTG 2174  
  
2515 TCTTCATCATCCTGCTGGTGACCTACATCATCCTCACCTTTGTGCTGCTCCTCAACATGC 2574  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2175 TCTTCATCATCCTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGC 2234  
  
2575 TCATTGCCCTCATGGGCGAGACAGTGGGCCAGGTCTCCAAGGAGAGCAAGCACATCTGGA 2634  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2235 TCATCGCCCTCATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGA 2294  
  
2635 AGCTGCAGTGGGCCACCACCATCCTGGACATTGAGCGCTCCTTCCCCGTATTCTGAGGA 2694  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2295 AGCTGCAGAGAGCCATCACCATCCTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGA 2354  
  
2695 AGGCCTTCCGCTCTGGGGAGATGGTCACCGTGGGCAAGAGCTCGGACGGCACTCCTGACC 2754  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2355 AGGCCTTCCGCTCAGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACT 2414  
  
2755 GCAGGTGGTGCTTCAGGGTGGATGAGGTGAACTGGTCTCACTGGAACCAGAACTTGGGCA 2814  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2415 ACCGGTGGTGCTTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCA 2474  
  
2815 TCATCAACGAGGACCCGGGCAAGAATGAGACCTACCAGTATTATGGCTTCTCGCATACCG 2874  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2475 TCATCAACGAAGACCCGGGCAACTGTGAGGGCGTCAAG...CGCACCTGAGCTTCTCC 2530  
  
2875 TGGGCCGCCTCCGCAGGGATCGCTGGTCCTCGGTGGTACCCGCGTGGTGGAACCTGAACA 2934  
| | | | | | | | | | | | | | | | | | | | | | | | | | | |  
2531 CTGCGGTCAAGCAGAGTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTA 2590

**SUBSTITUTE SHEET (RULE 26)**

2935 AGAACTCGAACCCGGACGAGGTGGTGGTGCCTCTGGACAGCATGGGGAACCCCCGCTGCG 2994  
||| || | | | | | | | | | | | |  
2591 AGA...GAGGCAAGTGCTCGAGATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCG 2646

2995 ATGGCCACCAGCAGGGTTACCCCCGCAAGTGGAGGACTGATGACGCCCCGCTCTAGGGAC 3054  
| | | | | | | | | | | | | | | |  
2647 A...CAGTTTTTCAGGGTCTCTGAAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGC 2703

3055 TGCAGCCCAGCCCCAGCTTCTCTGCCACTCATTTCTAGTCCAGCCGCATTTTCAGCAGTG 3114  
| | | | | | | | | | | | | | | |  
2704 TTCCGGGGAGAAGTGAGGACGTCACGCAGACAGCACTGTCAACACTGGGCCTTAGGAGAC 2763

3115 CCTTCTGGGGTGTCCCCCACACCCTGCTTTGGCCCCAGAGGCGAGGGACCAGTGGAGGT 3174  
|| | | | | | | | | | | | | | |  
2764 CC.....CGTTGCCACGGGGGGCTTGCTGAGGGAACACCAGTGCTCTGTCAGCAGCCTG 2817

3175 GCCAGGGAGGCCCCAGGACCCTGTGGTCCCCTGGCTCTGCCTCCCCA.CCCTGGGGTGGG 3233  
||| || | | | | | | | | | | | | | |  
2818 GCCTGGTCTGTGCCTGCCAGCATGTTCCCAAATCTGTGCTGGACAAGCTGTGGGAAGCG 2877

3234 GGCTCCCGGCCACCTGTCTTGCTCCTATGGA...GTCACATAAGCCAACGCCAGAGCCCC 3290  
|| | | | | | | | | | | | | | |  
2878 TTCTTGGAAGCATGGGGAGTGATGACATCCAACCGTCACTGTCCCAAGTGAATCTCCTAC 2937

3291 TCCACCTCAGGCCCCAGCCCCTGCCTCTCCATTATTTATTTGCTCTGCTCTCAGGAAGCG 3350  
|||| | | | | | | | | | | | |  
2938 AGACTTTCAGGTTTTACTACTTAACAGTTGGATGGCAGCTCTACTGGGACATGTAG 2997

335I ACGTGACCCCTGCCCCAGCTGGAACCTGGCAGAGGCCTTAGGACCCCGTTCCAAGTGCAC 3410  
| | | | | | | | | | | | | | | |  
2998 GCCTTGGTTCCTTGATTAATCTTTTTTTGAACGAATTACTTTTACCAGCTGATGATGGC.. 3055

**FIG. 5E**

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```

1  MADSSSEGPRAGPGEVAELPGDESGTPGGEAFPLSSLANLFEGEDGSLSPSPADASRPAGP 60
1  .....MKKWSSTDLGAAADPLQKDTCPDPLDGDPNRP 33
61  GDGRPNL.....RMKFQGAFRKGVPNPIDLLESTLYESSVVPGPKKAPMDSLFDYGTyrH 115
    :| | | | | :| | | | | :| | | | | | | | | | | | | | | |
34  PPAKPQLSTAKSRTLFGKGDSEEAFFVDCPHEE.GELDSCPTITVSPVITI.....Q 85
116  HSSDNKRWRKKIIEKQPQSPKAPAPPPPILKVFNRPIILFDIVSRGSTADLDGLLPFLT 175
    | | | | | : | | | | | | | | | | | | | | | | | | | | | |
86  RPGDGP TGARLL.....SQDSVAASTEKTLRLYDRRSIFEAVAQNNCQDLESLLLFLQK 139
176  HKKRLTDEEFREPSTGKTCLPKALLNLSNGRNDTIPVLLDIAERTGNMREFINSPFRDIY 235
    || ||| ||| :| | | | | ||| :| | | | | .| | | | | ||| :| | | | | .| :| | :| | | |
140  SKKHLTDNEFKDPETGKTCLLKAMLNLDHGQNTTIPLLLEIARQTDSLKELVNASYTDSY 199
236  YRGQTALHIAIERRCKHYVELLVAQGADVHAQARGRFFQPKDEGGYFYFGELPLSLAACT 295
    |:| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
200  YKGQTALHIAIERRNMALVTLLVENGADVQAAAHGDDFFKTKGRPGFYFGELPLSLAACT 259
296  NQPHIVNYLTENPHKKADMRRQDSRGNTVLHALVAIADNTRENTKFVTKMYDLLLLKCAR 355
    || || | :| :| | | | | | | | | | | | | | | | | | | | | | | | | |
260  NQLGIVKFLQNSWQTADISARDSVGNTVLHALVEVADNTADNTKFVTSMYNEILILGAK 319
356  LFPDSNLEAVLNNDGOLSPLMMAAKTGKIGIFQHIIRREVTDTRHLSRKFKDWAYGPVY 415
    | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
320  LHPTLKLEELTNKKGMTPLALAAGTGKIGVLAYILQREIQEPCRHLRSKFTWAYGPVH 379
416  SSLYDLSSLDTCGEEASVLEILVY.NSKIENRHEMLAVEPINELLRDKWRKFGAVSFYIN 474
    ||| ||| :| | | | | | | | | | | | | | | | | | | | | | | | | | | |
380  SSLYDLSCIDTC.EKNSVLEVIAYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFN 438
475  VVSYLCAMVIFTLTAYYQPLEGTPPYPYRTTVDYLRRLAGEVITLFTGVLFFFTNIKDLFM 534
    . | | | | | :| | | | | | | | | | | | | | | | | | | | | | | | | | | |
439  FLVYCLYMIIFTMAAYYRPVDGLPPFKMEKTGDYFRVTGEILSVLGGVYFFFRGIQ.YFL 497

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FIG.6A

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535 KKCPGVNSLFIDGSFQLLYFIYSVLVIVSAALYLAGIEAYLAVMVFALVLGWMNALYFTR 594  
 .: | . . ||:| :.:|:|: |. .| . || . :. |. | ||. | || | ||:|  
 498 QRRPSMKTLFVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSLALGWTNMLYYTR 557  
 595 GLKLTGTYSIMIQKILFKDLFRFLLVYLLFMIGYASALVSLLNPCANMKVCNEDQTNCTV 654  
 | . | |.:||:|. : || ||: ||:|. : |.:|. |.: | . . | ..  
 558 GFQQMGIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLIEDGKNDSLPESTSHRW. 616  
 655 PTYPSCR..DSETFSTF..LLDLFKLTIGMGDLEMLSSTKYPVVFIIILLVTYIILTFVLL 710  
 |. || || | : |: || | || || || | . : || || ||. |: || |: ||  
 617 .RGPACRPPDSSYNSLYSTCLELFKFTIGMGDLEFTENYDFKAVFIILLLAYVILTYILL 675  
 711 LNMLIALMGETVGQVSKEKHIWKLQWATTILDIERSFVPVFLRKAFRSGEMVTVGKSSDG 770  
 || || || || || | :. . ||. || || | || || |: || : || || ||. :. || . ||  
 676 LNMLIALMGETVNKIAQESKNIWKLQRAITILDTEKSFLKCMRKAFRSGKLLQVGYPDPG 735  
 771 TPDRRWCFRVDEVNWSHWNQNLGIINEDPGKNE.TYQYYGFSHTVGRLRRDRWS..SVVP 827  
 | || || || || ||. || | || || || | . || |. | . ||  
 736 KDDYRWCFRVDEVNWTWNTNVGIINEDPGNCEGVKRTLFSLSRVSGRHWKNFALVP 795  
 828 RVVELN....KNSNPDEVVV.....PLDS..MGNPRCDGHQQGYPRKWRTDDAPL\* 872  
 . | . ... |: || . | |. . | | .  
 796 LLREASARDRQSAQPEEVYLRQFSGSLKPEDAEVFKSPAASGEK\*..... 840

FIG.6B

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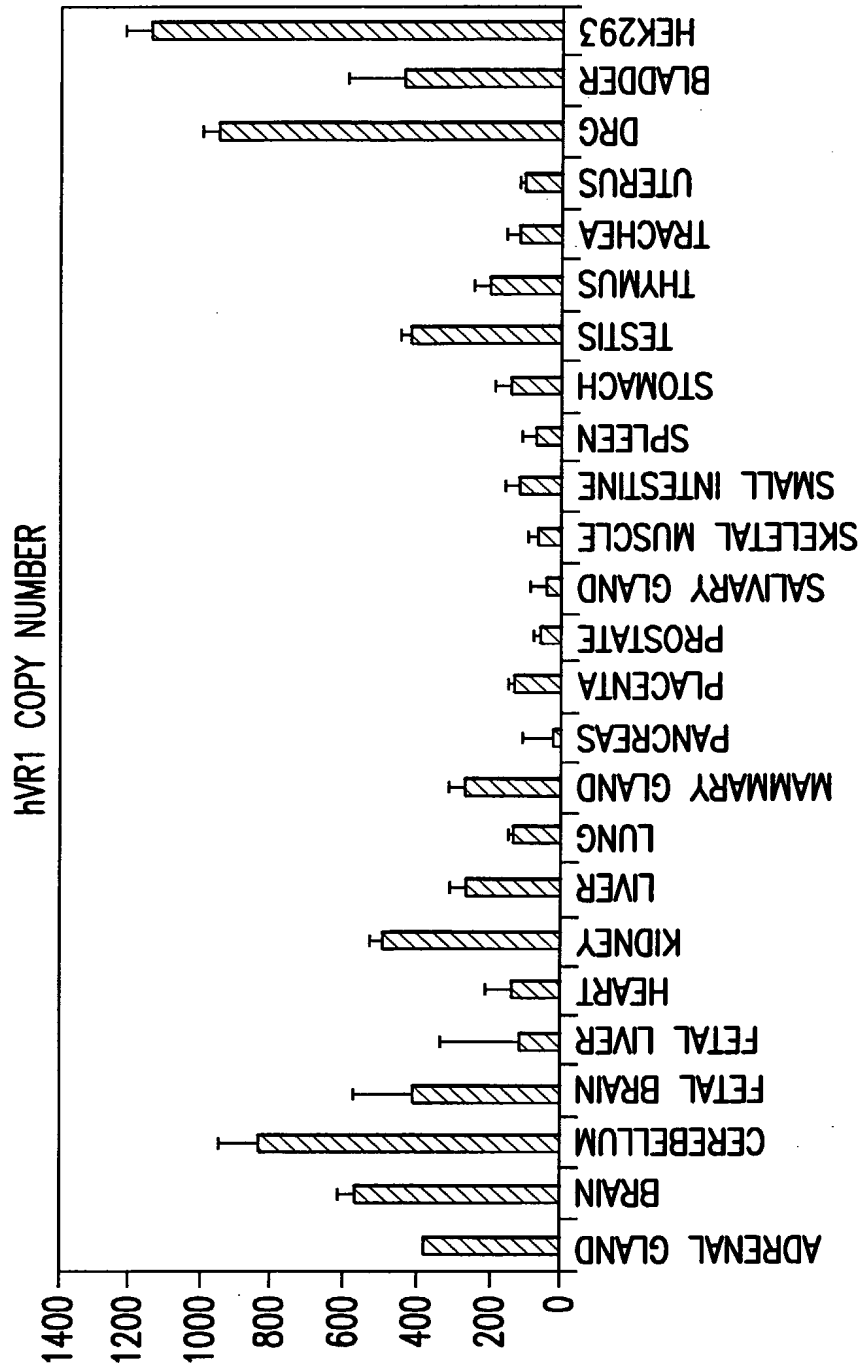


FIG.7A

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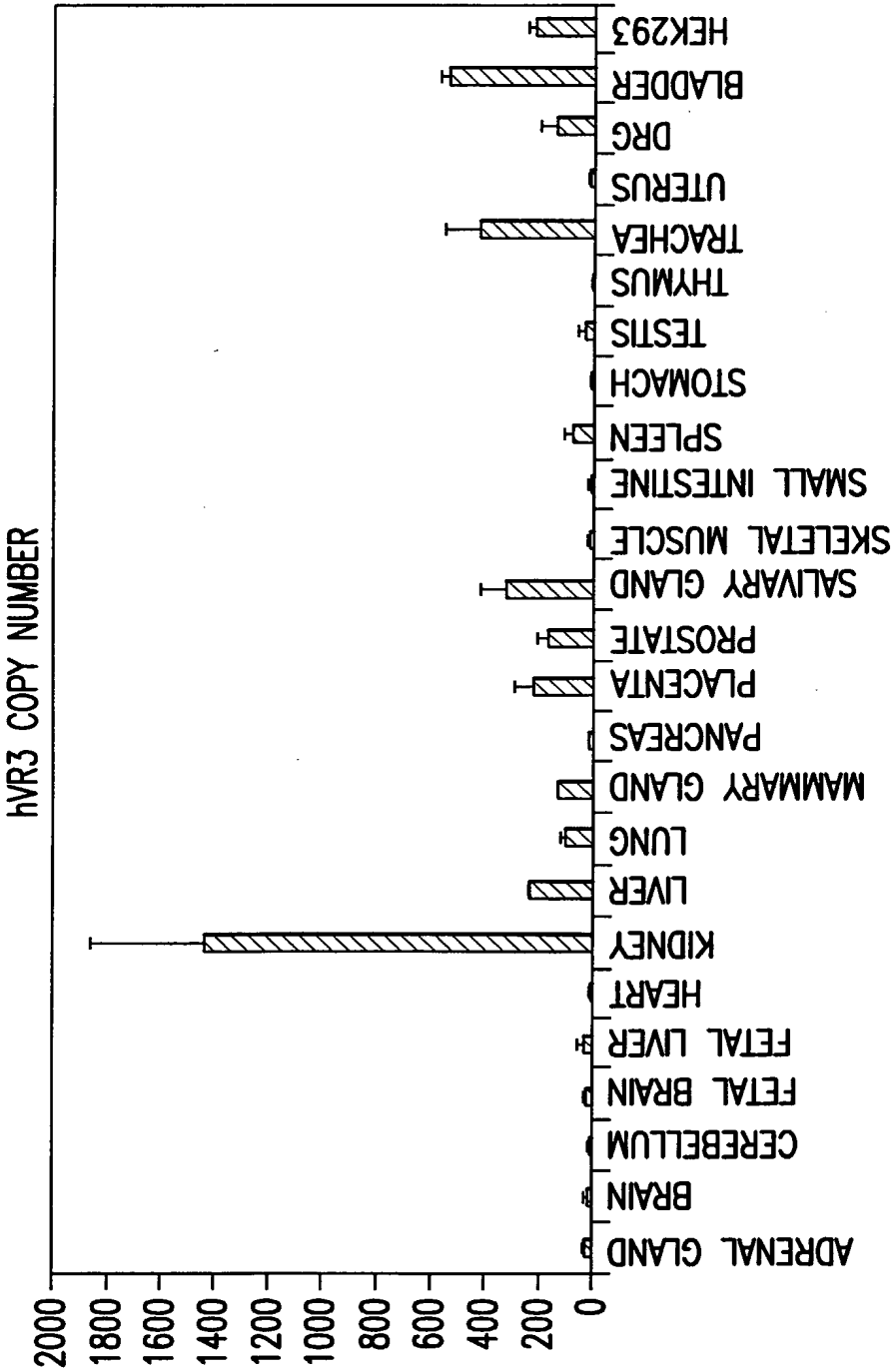


FIG.7B



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gagccaccca	ctgctccagc	ctgccccagc	tggtccctct	gtctgtcctc	tctgttttgc	60
agatggggaa	actgaggctt	aggtcgggga	tctagacaat	tgggatttaa	acccagggac	120
tatccagccc	caaagccctt	cccaccacac	caggtggcct	gtcctggggc	cagctctgca	180
cacagggcct	ggtgcccccg	gggtgcttgg	gaagtggcag	ggcagaggtg	ggccctgtgg	240
ctgttctggc	tcagcttcta	aaacaagagc	ctctgctggg	ggcagagggg	ccgtgaaccc	300
ctgaaatgtt	aggcagatac	cctgtgggag	ctttgttctg	ggatgctaag	aaccgcttga	360
ggatttaagc	tttgccactt	tggctccgga	gcaagggcag	agggtgagc	agtgcagacg	420
ggcctggggc	aggc atg gcg gat tcc agc gaa ggc ccc cgc gcg ggg ccc	Met Ala Asp Ser Ser Glu Gly	Pro Arg Ala Gly Pro			470
	1		5		10	
ggg gag gtg gct gag ctc ccc ggg gat gag agt ggc acc cca ggt ggg						518
Gly Glu Val Ala Glu Leu Pro Gly Asp Glu Ser Gly Thr Pro Gly Gly	15		20		25	
gag gct ttt cct ctc tcc tcc ctg gcc aat ctg ttt gag ggg gag gat						566
Glu Ala Phe Pro Leu Ser Ser Leu Ala Asn Leu Phe Glu Gly Glu Asp	30		35		40	
ggc tcc ctt tcg ccc tca ccg gct gat gcc agt cgc cct gct ggc cca						614
Gly Ser Leu Ser Pro Ser Pro Ala Asp Ala Ser Arg Pro Ala Gly Pro	45		50		55	60
ggc gat ggg cga cca aat ctg cgc atg aag ttc cag ggc gcc ttc cgc						662
Gly Asp Gly Arg Pro Asn Leu Arg Met Lys Phe Gln Gly Ala Phe Arg		65		70		75
aag ggg gtg ccc aac ccc atc gat ctg ctg gag tgg acc cta tat gag						710
Lys Gly Val Pro Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu		80		85		90
tcc tcg gtg gtg cct ggg ccc aag aaa gca ccc atg gac tca ctg ttt						758
Ser Ser Val Val Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe		95		100		105
gac tac ggc acc tat cgt cac cac tcc agt gac aac aag agg tgg agg						806
Asp Tyr Gly Thr Tyr Arg His His Ser Ser Asp Asn Lys Arg Trp Arg		110		115		120
aag aag atc ata gag aag cag ccg cag agc ccc aaa gcc cct gcc cct						854
Lys Lys Ile Ile Glu Lys Gln Pro Gln Ser Pro Lys Als Pro Ala Pro			130		130	140
cag ccg ccc ccc atc ctc aaa gtc ttc aac cgg cct atc ctc ttt gac						902
Gln Pro Pro Pro Ile Leu Lys Val Phe Asn Arg Pro Ile Leu Phe Asp		145		150		155
atc gtg tcc cgg ggc tcc act gct gac ctg gac ggg ctg ctc cca ttc						950
Ile Val Ser Arg Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Pro Phe		160		165		170

FIG.8A

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ttg	ctg	acc	cac	aag	aaa	cgc	cta	act	gat	gag	gag	ttt	cga	gag	cca	998
Leu	Leu	Thr 175	His	Lys	Lys	Arg	Leu 180	Thr	Asp	Glu	Glu	Phe 185	Arg	Glu	Pro	
tct	acg	ggg	aag	acc	tgc	ctg	ccc	agg	gcc	ttg	ctg	aac	ctg	agc	aat	1046
Ser	Thr 190	Gly	Lys	Thr	Cys	Leu 195	Pro	Lys	Ala	Leu	Leu 200	Asn	Leu	Ser	Asn	
ggc	cgc	aac	gac	acc	atc	cct	gtg	ctg	ctg	gac	atc	gcg	gag	cgc	acc	1094
Gly	Arg	Asn	Asp	Thr	Ile 210	Pro	Val	Leu	Leu	Asp 215	Ile	Ala	Glu	Arg	Thr 220	
ggc	aac	atg	cgg	gag	ttc	att	aac	tcg	ccc	ttc	cgt	gac	atc	tac	tat	1142
Gly	Asn	Met	Arg	Glu 225	Phe	Ile	Asn	Ser	Pro 230	Phe	Arg	Asp	Ile	Tyr 235	Tyr	
cga	ggt	cag	aca	gcc	ctg	cac	atc	gcc	att	gag	cgt	cgc	tgc	aaa	cac	1190
Arg	Gly	Gln	Thr 240	Ala	Leu	His	Ile	Ala 245	Ile	Glu	Arg	Arg	Cys 250	Lys	His	
tac	gtg	gaa	ctt	ctc	gtg	gcc	cag	gga	gct	gat	gtc	cac	gcc	cag	gcc	1238
Tyr	Val	Glu 255	Leu	Leu	Val	Ala	Gln 260	Gly	Ala	Asp	Val	His 265	Ala	Gln	Ala	
cgt	ggg	cgc	ttc	ttc	cag	ccc	aag	gat	gag	ggg	ggc	tac	ttc	tac	ttt	1286
Arg	Gly 270	Arg	Phe	Phe	Gln	Pro 275	Lys	Asp	Glu	Gly	Gly 280	Tyr	Phe	Tyr	Phe	
ggg	gag	ctg	ccc	ctg	tcg	ctg	gct	gcc	tgc	acc	aac	cag	ccc	cac	att	1334
Gly	Glu	Leu	Pro	Leu	Ser 290	Leu	Ala	Ala	Cys	Thr 295	Asn	Gln	Pro	His	Ile 300	
gtc	aac	tac	ctg	acg	aga	aac	ccc	cac	aag	aag	gcg	gac	atg	cgg	cgc	1382
Val	Asn	Tyr	Leu	Thr 305	Glu	Asn	Pro	His	Lys 310	Lys	Ala	Asp	Met	Arg 315	Arg	
cag	gac	tcg	cga	ggc	aac	aca	gtg	ctg	cat	gcg	ctg	gtg	gcc	att	gct	1430
Gln	Asp	Ser	Arg 320	Gly	Asn	Thr	Val	Leu 325	His	Ala	Leu	Val	Ala 330	Ile	Ala	
gac	aac	acc	cgt	gag	aac	acc	aag	ttt	gtt	acc	aag	atg	tac	gac	ctg	1478
Asp	Asn	Thr 335	Arg	Glu	Asn	Thr	Lys	Phe	Val	Thr	Lys	Met 345	Tyr	Asp	Leu	
ctg	ctg	ctc	aag	tgt	gcc	cgc	ctc	ttc	ccc	gac	agc	aac	ctg	gag	gcc	1526
Leu	Leu	Leu	Lys	Cys	Ala	Arg 355	Leu	Phe	Pro	Asp	Ser	Asn	Leu	Glu	Ala	
gtg	ctc	aac	aac	gac	ggc	ctc	tcg	ccc	ctc	atg	atg	gct	gcc	aag	acg	1574
Val	Leu	Asn	Asn	Asp	Gly 370	Leu	Ser	Pro	Leu	Met 375	Met	Ala	Ala	Lys	Thr 380	

FIG.8B

21/23

ggc Gly	aag Lys	att Ile	ggg Gly	atc Ile 385	ttt Phe	cag Gln	cac His	atc Ile 390	atc Ile 390	cgg Arg	cgg Arg	gag Glu	gtg Val	acg Thr 395	gat Asp	1622
gag Glu	gac Asp	aca Thr	cgg Arg 400	cac His	ctg Leu	tcc Ser	cgc Arg	aag Lys 405	ttc Phe	aag Lys	gac Asp	tgg Trp	gcc Ala 410	tat Tyr	ggg Gly	1670
cca Pro	gtg Val	tat Tyr 415	tcc Ser	tcg Ser	ctt Leu	tat Tyr	gac Asp 420	ctc Leu	tcc Ser	tcc Ser	ctg Leu	gac Asp 425	acg Thr	tgt Cys	ggg Gly	1718
gaa Glu	gag Glu 430	gcc Ala	tcc Ser	gtg Val	ctg Leu	gag Glu 435	atc Ile	ctg Leu	gtg Val	tac Tyr	aac Asn 440	agc Ser	aag Lys	att Ile	gag Glu	1766
aac Asn 445	cgc Arg	cac His	gag Glu	atg Met	ctg Leu 450	gct Ala	gtg Val	gag Glu	ccc Pro	atc Ile 455	aat Asn	gaa Glu	ctg Leu	ctg Leu	cgg Arg 460	1814
gac Asp	aag Lys	tgg Trp	cgc Arg	aag Lys 465	ttc Phe	ggg Gly	gcc Ala	gtc Val	tcc Ser 470	ttc Phe	tac Tyr	atc Ile	aac Asn	gtg Val 475	gtc Val	1862
tcc Ser	tac Tyr	ctg Leu	tgt Cys 480	gcc Ala	atg Met	gtc Val	atc Ile	ttc Phe 485	act Thr	ctc Leu	acc Thr	gcc Ala	tac Tyr 490	tac Tyr	cag Gln	1910
ccg Pro	ctg Leu	gag Glu 495	ggc Gly	aca Thr	ccg Pro	ccg Pro	tac Tyr 500	cct Pro	tac Tyr	cgc Arg	acc Thr	acg Thr 505	gtg Val	gac Asp	tac Tyr	1958
ctg Leu 510	cgg Arg	ctg Leu	gct Ala	ggc Gly	gag Glu	gtc Val 515	att Ile	acg Thr	ctc Leu	ttc Phe	act Thr 520	ggg Gly	gtc Val	ctg Leu	ttc Phe	2006
ttc Phe 525	ttc Phe	acc Thr	aac Asn	atc Ile	aaa Lys 530	gac Asp	ttg Leu	ttc Phe	atg Met	aag Lys 535	aaa Lys	tgc Cys	cct Pro	gga Gly	gtg Val 540	2054
aat Asn	tct Ser	ctc Leu	ttc Phe	att Ile 545	gat Asp	ggc Gly	tcc Ser	ttc Phe	cag Gln 550	ctg Leu	ctc Leu	tac Tyr	ttc Phe	atc Ile 555	tac Tyr	2102
tct Ser	gtc Val	ctg Leu	gtg Val 560	atc Ile	gtc Val	tca Ser	gca Ala	gcc Ala 565	ctc Leu	tac Tyr	ctg Leu	gca Ala	ggg Gly 570	atc Ile	gag Glu	2150
gcc Ala	tac Tyr	ctg Leu 575	gcc Ala	gtg Val	atg Met	gtc Val	ttt Phe 580	gcc Ala	ctg Leu	gtc Val	ctg Leu	ggc Gly 585	tgg Trp	atg Met	aat Asn	2198

FIG.8C

22/23

gcc Ala	ctt Leu	tac Tyr	ttc Phe	acc Thr	cgt Arg	ggg Gly	ctg Leu	aag Lys	ctg Leu	acg Thr	ggg Gly	acc Thr	tat Tyr	agc Ser	atc Ile	2246
590						595					600					
atg Met	atc Ile	cag Gly	aag Lys	att Ile	ctc Leu	ttc Phe	aag Lys	gac Asp	ctt Leu	ttc Phe	cga Arg	ttc Phe	ctg Leu	ctc Leu	gtc Val	2294
605					610					615					620	
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				625					630					635		
aac Asn	ccg Pro	tgt Cys	gcc Ala	aac Asn	atg Met	aag Lys	gtg Val	tgc Cys	aat Asn	gag Glu	gac Asp	cag Gln	acc Thr	aag Asn	tgc Cys	2390
			640					645					650			
aca Thr	gtg Val	ccc Pro	act Thr	tac Tyr	ccc Pro	tcg Ser	tgc Cys	cgt Arg	gac Asp	agc Ser	gag Glu	acc Thr	ttc Phe	agc Ser	acc Thr	2438
		655					660					665				
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670						675					680					
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685					690					695					700	
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		720					725						730			
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		735					740					745				
ccc Pro	gta Val	ttc Phe	ctg Leu	agg Arg	aag Lys	gcc Ala	ttc Phe	cgc Arg	tct Ser	ggg Gly	gag Glu	atg Met	gtc Val	acc Thr	gtg Val	2726
	750					755					760					
ggc Gly	aag Lys	agc Ser	tcg Ser	gac Asp	ggc Gly	act Thr	cct Pro	gac Asp	cgc Arg	agg Arg	tgg Trp	tgc Cys	ttc Phe	agg Arg	gtg Val	2774
765					770					775					780	
gat Asp	gag Glu	gtg Val	aac Asn	tgg Trp	tct Ser	cac His	tgg Trp	aac Asn	cag Gln	aac Asn	ttg Leu	ggc Gly	atc Ile	atc Ile	aac Asn	2822
				785					790					795		

FIG.8D

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gag gac ccg ggc aag aat gag acc tac cag tat tat ggc ttc tcg cat	2870
Glu Asp Pro Gly Lys Asn Glu Thr Tyr Gln Tyr Tyr Gly Phe Ser His	
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acc gtg ggc cgc ctc cgc agg gat cgc tgg tcc tcg gtg gta ccc cgc	2918
Thr Val Gly Arg Leu Arg Arg Asp Arg Trp Ser Ser Val Val Pro Arg	
815 820 825	
gtg gtg gaa ctg aac aag aac tcg aac ccg gac gag gtg gtg gtg cct	2966
Val Val Glu Leu Asn Lys Ser Asn Pro Asp Glu Val Val Val Pro	
830 835 840	
ctg gac agc atg ggg aac ccc cgc tgc gat ggc cac cag cag ggt tac	3014
Leu Asp Ser Met Gly Asn Pro Arg Cys Asp Gly His Gln Gln Gly Tyr	
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Pro Arg Lys Trp Arg Thr Asp Asp Ala Pro Leu *	
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FIG.8E

## SEQUENCE LISTING

<110> Abbott Laboratories  
Masters, Jeffrey N.  
Vos, Melissa H.

<120> Human Vanilloid Receptor Gene

<130> 6430.US.P1

<140> US 09/438,997

<141> 1999-11-12

<150> US 09/191,139

<151> 1998-11-13

<160> 16

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 3599

<212> DNA

<213> Homo sapiens

<400> 1

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&lt;211&gt; 871

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

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Met Ala Asp Ser Ser Glu Gly Pro Arg Ala Gly Pro Gly Glu Val Ala
1          5          10          15
Glu Leu Pro Gly Asp Glu Ser Gly Thr Pro Gly Gly Glu Ala Phe Pro
20        25        30
Leu Ser Ser Leu Ala Asn Leu Phe Glu Gly Glu Asp Gly Ser Leu Ser
35        40        45
Pro Ser Pro Ala Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg
50        55        60
Pro Asn Leu Arg Met Lys Phe Gln Gly Ala Phe Arg Lys Gly Val Pro
65        70        75        80
Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val Val
85        90        95
Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr
100       105       110
Tyr Arg His His Ser Ser Asp Asn Lys Arg Trp Arg Lys Lys Ile Ile
115       120       125
Glu Lys Gln Pro Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro
130       135       140
Ile Leu Lys Val Phe Asn Arg Pro Ile Leu Phe Asp Ile Val Ser Arg
145       150       155       160
Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Pro Phe Leu Leu Thr His
165       170       175
Lys Lys Arg Leu Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys
180       185       190

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Thr	Cys	Leu	Pro	Lys	Ala	Leu	Leu	Asn	Leu	Ser	Asn	Gly	Arg	Asn	Asp
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Thr	Ile	Pro	Val	Leu	Leu	Asp	Ile	Ala	Glu	Arg	Thr	Gly	Asn	Met	Arg
		210				215					220				
Glu	Phe	Ile	Asn	Ser	Pro	Phe	Arg	Asp	Ile	Tyr	Tyr	Arg	Gly	Gln	Thr
225					230					235					240
Ala	Leu	His	Ile	Ala	Ile	Glu	Arg	Arg	Cys	Lys	His	Tyr	Val	Glu	Leu
				245					250					255	
Leu	Val	Ala	Gln	Gly	Ala	Asp	Val	His	Ala	Gln	Ala	Arg	Gly	Arg	Phe
			260					265					270		
Phe	Gln	Pro	Lys	Asp	Glu	Gly	Gly	Tyr	Phe	Tyr	Phe	Gly	Glu	Leu	Pro
		275					280					285			
Leu	Ser	Leu	Ala	Ala	Cys	Thr	Asn	Gln	Pro	His	Ile	Val	Asn	Tyr	Leu
		290				295					300				
Thr	Glu	Asn	Pro	His	Lys	Lys	Ala	Asp	Met	Arg	Arg	Gln	Asp	Ser	Arg
305					310					315					320
Gly	Asn	Thr	Val	Leu	His	Ala	Leu	Val	Ala	Ile	Ala	Asp	Asn	Thr	Arg
				325					330					335	
Glu	Asn	Thr	Lys	Phe	Val	Thr	Lys	Met	Tyr	Asp	Leu	Leu	Leu	Leu	Lys
			340					345					350		
Cys	Ala	Arg	Leu	Phe	Pro	Asp	Ser	Asn	Leu	Glu	Ala	Val	Leu	Asn	Asn
		355					360					365			
Asp	Gly	Leu	Ser	Pro	Leu	Met	Met	Ala	Ala	Lys	Thr	Gly	Lys	Ile	Gly
		370				375					380				
Ile	Phe	Gln	His	Ile	Ile	Arg	Arg	Glu	Val	Thr	Asp	Glu	Asp	Thr	Arg
385					390					395					400
His	Leu	Ser	Arg	Lys	Phe	Lys	Asp	Trp	Ala	Tyr	Gly	Pro	Val	Tyr	Ser
				405					410					415	
Ser	Leu	Tyr	Asp	Leu	Ser	Ser	Leu	Asp	Thr	Cys	Gly	Glu	Glu	Ala	Ser
			420					425					430		
Val	Leu	Glu	Ile	Leu	Val	Tyr	Asn	Ser	Lys	Ile	Glu	Asn	Arg	His	Glu
		435					440					445			
Met	Leu	Ala	Val	Glu	Pro	Ile	Asn	Glu	Leu	Leu	Arg	Asp	Lys	Trp	Arg
		450				455					460				
Lys	Phe	Gly	Ala	Val	Ser	Phe	Tyr	Ile	Asn	Val	Val	Ser	Tyr	Leu	Cys
465					470					475					480
Ala	Met	Val	Ile	Phe	Thr	Leu	Thr	Ala	Tyr	Tyr	Gln	Pro	Leu	Glu	Gly
				485					490					495	
Thr	Pro	Pro	Tyr	Pro	Tyr	Arg	Thr	Thr	Val	Asp	Tyr	Leu	Arg	Leu	Ala
			500					505					510		
Gly	Glu	Val	Ile	Thr	Leu	Phe	Thr	Gly	Val	Leu	Phe	Phe	Phe	Thr	Asn
		515					520					525			
Ile	Lys	Asp	Leu	Phe	Met	Lys	Lys	Cys	Pro	Gly	Val	Asn	Ser	Leu	Phe
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Ile															

[illegible]

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<210> 4
<211> 838
<212> PRT
<213> Rattus norvegicus
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Gln	Glu	Asn	Ser	Cys	Leu	Asp	Pro	Pro	Asp	Arg	Asp	Pro	Asn	Cys	Lys	
		20						25					30			
Pro	Pro	Pro	Val	Lys	Pro	His	Ile	Phe	Thr	Thr	Arg	Ser	Arg	Thr	Arg	
		35					40					45				
Leu	Phe	Gly	Lys	Gly	Asp	Ser	Glu	Glu	Ala	Ser	Pro	Leu	Asp	Cys	Pro	
	50				55						60					
Tyr	Glu	Glu	Gly	Gly	Leu	Ala	Ser	Cys	Pro	Ile	Ile	Thr	Val	Ser	Ser	
65				70					75						80	
Val	Leu	Thr	Ile	Gln	Arg	Pro	Gly	Asp	Gly	Pro	Ala	Ser	Val	Arg	Pro	
				85					90					95		
Ser	Ser	Gln	Asp	Ser	Val	Ser	Ala	Gly	Glu	Lys	Pro	Pro	Arg	Leu	Tyr	
			100					105					110			
Asp	Arg	Arg	Ser	Ile	Phe	Asp	Ala	Val	Ala	Gln	Ser	Asn	Cys	Gln	Glu	
		115					120					125				
Leu	Glu	Ser	Leu	Leu	Pro	Phe	Leu	Gln	Arg	Ser	Lys	Lys	Arg	Leu	Thr	
	130					135					140					
Asp	Ser	Glu	Phe	Lys	Asp	Pro	Glu	Thr	Gly	Lys	Thr	Cys	Leu	Leu	Lys	
145				150					155						160	

Ala	Met	Leu	Asn	Leu	His	Asn	Gly	Gln	Asn	Asp	Thr	Ile	Ala	Leu	Leu		
				165					170						175		
Leu	Asp	Val	Ala	Arg	Lys	Thr	Asp	Ser	Leu	Lys	Gln	Phe	Val	Asn	Ala		
			180					185						190			
Ser	Tyr	Thr	Asp	Ser	Tyr	Tyr	Lys	Gly	Gln	Thr	Ala	Leu	His	Ile	Ala		
		195					200					205					
Ile	Glu	Arg	Arg	Asn	Met	Thr	Leu	Val	Thr	Leu	Leu	Val	Glu	Asn	Gly		
	210					215					220						
Ala	Asp	Val	Gln	Ala	Ala	Ala	Asn	Gly	Asp	Phe	Phe	Lys	Lys	Thr	Lys		
225				230						235					240		
Gly	Arg	Pro	Gly	Phe	Tyr	Phe	Gly	Glu	Leu	Pro	Leu	Ser	Leu	Ala	Ala		
				245					250					255			
Cys	Thr	Asn	Gln	Leu	Ala	Ile	Val	Lys	Phe	Leu	Leu	Gln	Asn	Ser	Trp		
		260						265					270				
Gln	Pro	Ala	Asp	Ile	Ser	Ala	Arg	Asp	Ser	Val	Gly	Asn	Thr	Val	Leu		
		275					280						285				
His	Ala	Leu	Val	Glu	Val	Ala	Asp	Asn	Thr	Val	Asp	Asn	Thr	Lys	Phe		
	290					295					300						
Val	Thr	Ser	Met	Tyr	Asn	Glu	Ile	Leu	Ile	Leu	Gly	Ala	Lys	Leu	His		
305					310					315					320		
Pro	Thr	Leu	Lys	Leu	Glu	Glu	Ile	Thr	Asn	Arg	Lys	Gly	Leu	Thr	Pro		
				325					330					335			
Leu	Ala	Leu	Ala	Ala	Ser	Ser	Gly	Lys	Ile	Gly	Val	Leu	Ala	Tyr	Ile		
			340					345					350				
Leu	Gln	Arg	Glu	Ile	His	Glu	Pro	Glu	Cys	Arg	His	Leu	Ser	Arg	Lys		
	355					360						365					
Phe	Thr	Glu	Trp	Ala	Tyr	Gly	Pro	Val	His	Ser	Ser	Leu	Tyr	Asp	Leu		
	370					375					380						
Ser	Cys	Ile	Asp	Thr	Cys	Glu	Lys	Asn	Ser	Val	Leu	Glu	Val	Ile	Ala		
385					390					395					400		
Tyr	Ser	Ser	Ser	Glu	Thr	Pro	Asn	Arg	His	Asp	Met	Leu	Leu	Val	Glu		
				405				410						415			
Pro	Leu	Asn	Arg	Leu	Leu	Gln	Asp	Lys	Trp	Asp	Arg	Phe	Val	Lys	Arg		
		420						425						430			
Ile	Phe	Tyr	Phe	Asn	Phe	Phe	Val	Tyr	Cys	Leu	Tyr	Met	Ile	Ile	Phe		
	435					440						445					
Thr	Ala	Ala	Ala	Tyr	Tyr	Arg	Pro	Val	Glu	Gly	Leu	Pro	Pro	Tyr	Lys		
	450					455					460						
Leu	Lys	Asn	Thr	Val	Gly	Asp	Tyr	Phe	Arg	Val	Thr	Gly	Glu	Ile	Leu		
465					470					475					480		
Ser	Val	Ser	Gly	Gly	Val	Tyr	Phe	Phe	Phe	Arg	Gly	Ile	Gln	Tyr	Phe		
				485					490					495			
Leu	Gln	Arg	Arg	Pro	Ser	Leu	Lys	Ser	Leu	Phe	Val	Asp	Ser	Tyr	Ser		
		500						505					510				
Glu	Ile	Leu	Phe	Phe	Val	Gln	Ser	Leu	Phe	Met	Leu	Val	Ser	Val	Val		
	515						520					525					
Leu	Tyr	Phe	Ser	Gln	Arg	Lys	Glu	Tyr	Val	Ala	Ser	Met	Val	Phe	Ser		
	530					535						540					
Leu	Ala	Met	Gly	Trp	Thr	Asn	Met	Leu	Tyr	Tyr	Thr	Arg	Gly	Phe	Gln		
545					550					555					560		
Gln	Met	Gly	Ile	Tyr	Ala	Val	Met	Ile	Glu	Lys	Met	Ile	Leu	Arg	Asp		
				565					570					575			
Leu	Cys	Arg	Phe	Met	Phe	Val	Tyr	Leu	Val	Phe	Leu	Phe	Gly	Phe	Ser		
		580						585					590				
Thr	Ala	Val	Val	Thr	Leu	Ile	Glu	Asp	Gly	Lys	Asn	Asn	Ser	Leu	Pro		
		595					600					605					
Met	Glu	Ser	Thr	Pro	His	Lys	Cys	Arg	Gly	Ser	Ala	Cys	Lys	Pro	Gly		

7

610		615		620
Asn Ser Tyr Asn Ser	Leu Tyr Ser Thr Cys	Leu Glu Leu Phe Lys Phe		
625	630	635		640
Thr Ile Gly Met Gly	Asp Leu Glu Phe Thr	Glu Asn Tyr Asp Phe Lys		
	645	650		655
Ala Val Phe Ile Ile	Leu Leu Leu Ala Tyr	Val Ile Leu Thr Tyr Ile		
	660	665		670
Leu Leu Leu Asn Met	Leu Ile Ala Leu Met	Gly Glu Thr Val Asn Lys		
	675	680		685
Ile Ala Gln Glu Ser	Lys Asn Ile Trp Lys	Leu Gln Arg Ala Ile Thr		
	690	695		700
Ile Leu Asp Thr Glu	Lys Ser Phe Leu Lys	Cys Met Arg Lys Ala Phe		
705	710	715		720
Arg Ser Gly Lys Leu	Leu Gln Val Gly Phe	Thr Pro Asp Gly Lys Asp		
	725	730		735
Asp Tyr Arg Trp Cys	Phe Arg Val Asp Glu	Val Asn Trp Thr Thr Trp		
	740	745		750
Asn Thr Asn Val Gly	Ile Ile Asn Glu Asp	Pro Gly Asn Cys Glu Gly		
	755	760		765
Val Lys Arg Thr Leu	Ser Phe Ser Leu Arg	Ser Gly Arg Val Ser Gly		
	770	775		780
Arg Asn Trp Lys Asn	Phe Ala Leu Val Pro	Leu Leu Arg Asp Ala Ser		
785	790	795		800
Thr Arg Asp Arg His	Ala Thr Gln Gln Glu	Glu Val Gln Leu Lys His		
	805	810		815
Tyr Thr Gly Ser Leu	Lys Pro Glu Asp Ala	Glu Val Phe Lys Asp Ser		
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Met Val Pro Gly Glu	Lys			
	835			

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 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 5  
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29

<210> 6  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 6  
 tatattgtcg acgtcctcac ttctccccg

29

<210> 7  
 <211> 2712  
 <212> DNA  
 <213> Homo sapiens

8,

&lt;400&gt; 7

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ccctaactcc aggccacctc cagccaagcc ccagctctcc acggccaaga gccgcacccg 180
gctctttggg aagggtgact cggaggaggc tttcccggtg gattgccctc acgaggaagg 240
tgagctggac tctgcccga ccatcacagt cagccctggt atcaccatcc agaggccagg 300
agacggcccc accggtgcca ggctgctgtc ccaggactct gtcgccgcca gcaccgagaa 360
gaccctcagg ctctatgatc gcaggagtat ctttgaagcc gttgctcaga ataactgcca 420
ggatctggag agcctgctgc tcttcttgca gaagagcaag aagcacctca cagacaacga 480
gttcaaagac cctgagacag ggaagacctg tctgctgaaa gccatgctca acctgcacga 540
cggacagaac accaccatcc ccctgctcct ggagatcgcg cggcaaacgg acagcctgaa 600
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cagattcgte aagcgcatct tctacttcaa ctctcctggt tactgcctgt acatgatcat 1380
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aagctgtggg aa 2712

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&lt;210&gt; 8

&lt;211&gt; 839

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 8

```

Met Lys Lys Trp Ser Ser Thr Asp Leu Gly Ala Ala Ala Asp Pro Leu
1           5           10           15

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Gln Lys Asp Thr Cys Pro Asp Pro Leu Asp Gly Asp Pro Asn Ser Arg  
 20 25 30  
 Pro Pro Pro Ala Lys Pro Gln Leu Ser Thr Ala Lys Ser Arg Thr Arg  
 35 40 45  
 Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro  
 50 55 60  
 His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro  
 65 70 75 80  
 Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu  
 85 90 95  
 Leu Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu  
 100 105 110  
 Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln  
 115 120 125  
 Asp Leu Glu Ser Leu Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu  
 130 135 140  
 Thr Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu  
 145 150 155 160  
 Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu  
 165 170 175  
 Leu Leu Glu Ile Ala Arg Gln Thr Asp Ser Leu Lys Glu Leu Val Asn  
 180 185 190  
 Ala Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile  
 195 200 205  
 Ala Ile Glu Arg Arg Asn Met Ala Leu Val Thr Leu Leu Val Glu Asn  
 210 215 220  
 Gly Ala Asp Val Gln Ala Ala Ala His Gly Asp Phe Phe Lys Lys Thr  
 225 230 235 240  
 Lys Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala  
 245 250 255  
 Ala Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser  
 260 265 270  
 Trp Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val  
 275 280 285  
 Leu His Ala Leu Val Glu Val Ala Asp Asn Thr Ala Asp Asn Thr Lys  
 290 295 300  
 Phe Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu  
 305 310 315 320  
 His Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr  
 325 330 335  
 Pro Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr  
 340 345 350  
 Ile Leu Gln Arg Glu Ile Gln Glu Pro Glu Cys Arg His Leu Ser Arg  
 355 360 365  
 Lys Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp  
 370 375 380  
 Leu Ser Cys Ile Asp Thr Cys Glu Lys Asn Ser Val Leu Glu Val Ile  
 385 390 395 400  
 Ala Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val  
 405 410 415  
 Glu Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys  
 420 425 430  
 Arg Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile  
 435 440 445  
 Phe Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe  
 450 455 460  
 Lys Met Glu Lys Thr Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu

10

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465                               470                               475                               480
Ser Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
                               485                               490                               495
Leu Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser
                               500                               505                               510
Glu Met Leu Phe Phe Leu Gln Ser Leu Phe Met Leu Ala Thr Val Val
                               515                               520                               525
Leu Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser
                               530                               535                               540
Leu Ala Leu Gly Trp Thr Asn Met Leu Tyr Tyr Thr Arg Gly Phe Gln
545                               550                               555                               560
Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp
                               565                               570                               575
Leu Cys Arg Phe Met Phe Val Tyr Ile Val Phe Leu Phe Gly Phe Ser
                               580                               585                               590
Thr Ala Val Val Thr Leu Ile Glu Asp Gly Lys Asn Asp Ser Leu Pro
                               595                               600                               605
Ser Glu Ser Thr Ser His Arg Trp Arg Gly Pro Ala Cys Arg Pro Pro
610                               615                               620
Asp Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys
625                               630                               635                               640
Phe Thr Ile Gly Met Gly Asp Leu Glu Phe Thr Glu Asn Tyr Asp Phe
                               645                               650                               655
Lys Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr
                               660                               665                               670
Ile Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn
675                               680                               685
Lys Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile
690                               695                               700
Thr Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala
705                               710                               715                               720
Phe Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys
                               725                               730                               735
Asp Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr
740                               745                               750
Trp Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu
755                               760                               765
Gly Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser
770                               775                               780
Gly Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala
785                               790                               795                               800
Ser Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg
                               805                               810                               815
Gln Phe Ser Gly Ser Leu Lys Pro Glu Asp Ala Glu Val Phe Lys Ser
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Pro Ala Ala Ser Gly Glu Lys
835

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&lt;210&gt; 9

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

11,

<400> 9  
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<210> 10  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 10  
tgatgaagac agccttgaag tca 23

<210> 11  
<211> 28  
<212> DNA  
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<220>  
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<400> 11  
agttctcagt gaactccagg tcgcccat 28

<210> 12  
<211> 20  
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<220>  
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<400> 12  
attgagaacc gccacgagat 20

<210> 13  
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<212> DNA  
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<220>  
<223> Primer

<400> 13  
agacggcccc gaacttg 17

<210> 14  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 14  
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12

<210> 15  
 <211> 764  
 <212> PRT  
 <213> Homo sapiens

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 <221> VARIANT  
 <222> (670)...(670)  
 <223> Xaa = Unknown or other at position 670

<400> 15  
 Met Thr Ser Pro Ser Ser Ser Pro Val Phe Arg Leu Glu Thr Leu Asp  
 1 5 10 15  
 Gly Gly Gln Glu Asp Gly Ser Glu Ala Asp Arg Gly Lys Leu Asp Phe  
 20 25 30  
 Gly Ser Gly Leu Pro Pro Met Glu Ser Gln Phe Gln Gly Glu Asp Arg  
 35 40 45  
 Lys Phe Ala Pro Gln Ile Arg Val Asn Leu Asn Tyr Arg Lys Gly Thr  
 50 55 60  
 Gly Ala Ser Gln Pro Asp Pro Asn Arg Phe Asp Arg Asp Arg Leu Phe  
 65 70 75 80  
 Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu  
 85 90 95  
 Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu  
 100 105 110  
 Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys  
 115 120 125  
 Asp Gly Val Asn Ala Cys Ile Leu Pro Leu Leu Gln Ile Asp Arg Asp  
 130 135 140  
 Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr  
 145 150 155 160  
 Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu  
 165 170 175  
 Gln Cys Val Lys Leu Leu Val Glu Asn Gly Ala Asn Val His Ala Arg  
 180 185 190  
 Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe  
 195 200 205  
 Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val  
 210 215 220  
 Val Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala  
 225 230 235 240  
 Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser  
 245 250 255  
 Asp Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly  
 260 265 270  
 Leu Leu Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp  
 275 280 285  
 Ile Arg Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu  
 290 295 300  
 Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly  
 305 310 315 320  
 Leu Ser His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val  
 325 330 335  
 Arg Val Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn  
 340 345 350  
 Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His

13

		355					360				365				
Arg	Met	Val	Val	Leu	Glu	Pro	Leu	Asn	Lys	Leu	Leu	Gln	Ala	Lys	Trp
	370					375					380				
Asp	Leu	Leu	Ile	Pro	Lys	Phe	Phe	Leu	Asn	Phe	Leu	Cys	Asn	Leu	Ile
385					390					395					400
Tyr	Met	Phe	Ile	Phe	Thr	Ala	Val	Ala	Tyr	His	Gln	Pro	Thr	Leu	Lys
				405					410					415	
Lys	Gln	Ala	Ala	Pro	His	Leu	Lys	Ala	Glu	Val	Gly	Asn	Ser	Met	Leu
			420					425					430		
Leu	Thr	Gly	His	Ile	Leu	Ile	Leu	Leu	Gly	Gly	Ile	Tyr	Leu	Leu	Val
		435					440					445			
Gly	Gln	Leu	Trp	Tyr	Phe	Trp	Arg	Arg	His	Val	Phe	Ile	Trp	Ile	Ser
	450					455					460				
Phe	Ile	Asp	Ser	Tyr	Phe	Glu	Ile	Leu	Phe	Leu	Phe	Gln	Ala	Leu	Leu
465					470					475					480
Thr	Val	Val	Ser	Gln	Val	Leu	Cys	Phe	Leu	Ala	Ile	Glu	Trp	Tyr	Leu
				485					490					495	
Pro	Leu	Leu	Val	Ser	Ala	Leu	Val	Leu	Gly	Trp	Leu	Asn	Leu	Leu	Tyr
		500						505					510		
Tyr	Thr	Arg	Gly	Phe	Gln	His	Thr	Gly	Ile	Tyr	Ser	Val	Met	Ile	Gln
	515						520					525			
Lys	Val	Ile	Leu	Arg	Asp	Leu	Leu	Arg	Phe	Leu	Leu	Ile	Tyr	Leu	Val
	530				535						540				
Phe	Leu	Phe	Gly	Phe	Ala	Val	Ala	Leu	Val	Ser	Leu	Ser	Gln	Glu	Ala
545					550					555					560
Trp	Arg	Pro	Glu	Ala	Pro	Thr	Gly	Pro	Asn	Ala	Thr	Glu	Ser	Val	Gln
				565					570					575	
Pro	Met	Glu	Gly	Gln	Glu	Asp	Glu	Gly	Asn	Gly	Ala	Gln	Tyr	Arg	Gly
		580						585					590		
Ile	Leu	Glu	Ala	Ser	Leu	Glu	Leu	Phe	Lys	Phe	Thr	Ile	Gly	Met	Gly
	595						600					605			
Glu	Leu	Ala	Phe	Gln	Glu	Gln	Leu	His	Phe	Arg	Gly	Met	Val	Leu	Leu
	610					615					620				
Leu	Leu	Leu	Ala	Tyr	Val	Leu	Leu	Thr	Tyr	Ile	Leu	Leu	Leu	Asn	Met
625					630					635					640
Leu	Ile	Ala	Leu	Met	Ser	Glu	Thr	Val	Asn	Ser	Val	Ala	Thr	Asp	Ser
				645					650					655	
Trp	Ser	Ile	Trp	Lys	Leu	Gln	Lys	Ala	Ile	Ser	Val	Leu	Xaa	Met	Glu
		660						665					670		
Asn	Gly	Tyr	Trp	Trp	Cys	Arg	Lys	Lys	Gln	Arg	Ala	Gly	Val	Met	Leu
	675						680					685			
Thr	Val	Gly	Thr	Lys	Pro	Asp	Gly	Ser	Pro	Asp	Glu	Arg	Trp	Cys	Phe
	690					695					700				
Arg	Val	Glu	Glu	Val	Asn	Trp	Ala	Ser	Trp	Glu	Gln	Thr	Leu	Pro	Thr
705					710					715					720
Leu	Cys	Glu	Asp	Pro	Ser	Gly	Ala	Gly	Val	Pro	Arg	Thr	Leu	Glu	Asn
				725					730					735	
Pro	Val	Leu	Ala	Ser	Pro	Pro	Lys	Glu	Asp	Glu	Asp	Gly	Ala	Ser	Glu
		740						745					750		
Glu	Asn	Tyr	Val	Pro	Val	Gln	Leu	Leu	Gln	Ser	Asn				
	755						760								

&lt;210&gt; 16

&lt;211&gt; 244

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

<220>

## <223> Consensus

<400> 16

[illegible]